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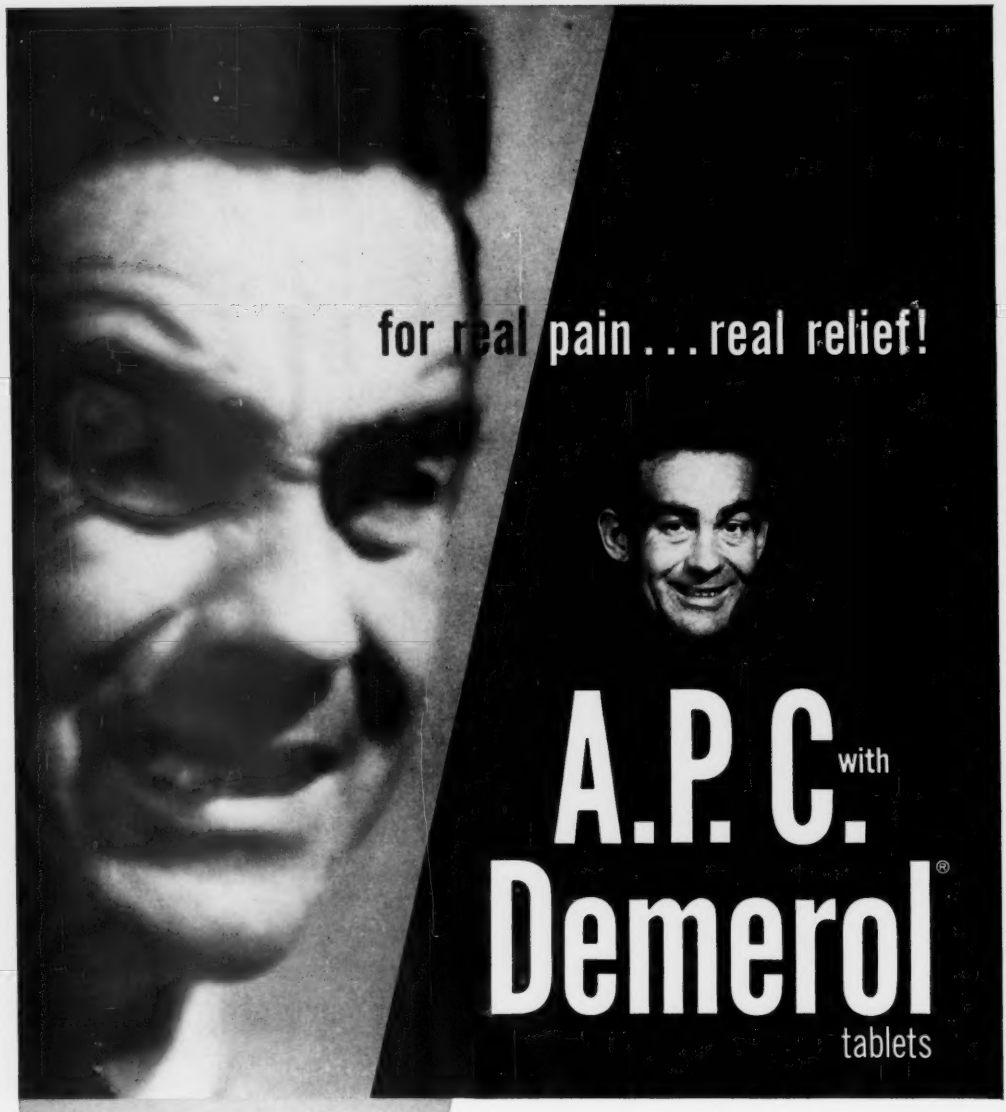

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THE NATURE OF DIETARY FAT AND THE PATTERN OF HEPATIC LIPOSIS IN CHOLINE-DEFICIENT MICE†

The amount of hepatic liposis that occurs in animals fed hypolipotropic diets is to some extent dependent upon the chain length and degree of saturation of the lipid components of the experimental diet.[10, 21]

This report is concerned with the relation of the chemical nature of the lipid components of a choline-deficient diet to the intralobular pattern of hepatic liposis. Lard, butter, or synthetic triglycerides served as the lipid components of the several diets. The major purpose of the experiments was accomplished in that two distinct patterns of hepatic liposis were produced, one a rapid and complete lobular liposis, the other much less extensive and predominantly restricted to peripheral (portal) zones of lobules.

MATERIALS AND METHODS

A diet that had previously produced hepatic liposis characteristic of choline deficiency in rats[15] and in mice[19] was used. The composition of this diet is as follows:

	gms.
Vitamin free casein	80.0
Sucrose	480.5
Fat (see table 1)	400.0
Salt Mixture (No. 2, U.S.P.XIII)	40.0
l-cystine	5.0
Cod liver oil	4.5
Vitamin powder	10.0

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The vitamin powder contains:

Thiamine hydrochloride	0.500
Riboflavin	0.250
Pyridoxine hydrochloride	0.200
Calcium pantothenate	1.000
Nicotinic acid	1.000
Powdered sugar	997.050

The duration of the experiments, the composition (lipids) of the 8 diets, and the number of mice fed each of the diets are shown in Table 1. Young adult mice of the C (Bagg albino) stock with an initial body weight of 20-22 gms. were used. From weaning until restriction to the high fat diets the mice were fed the standard laboratory ration of Purina Fox Chow.

In diet I (Table 1) lard or butter was used as the fat component. Of the 80 mice fed this diet only 15 received butter as the dietary fat. Five of these mice were killed after one day of feeding, 5 after 3-7 days, and 5 during the 10-30 days' interval. These mice showed a pattern of hepatic liposis identical to that observed in mice fed the diet containing lard. In the remaining diets (II-VIII) the dietary lipid was supplied as one, two, or three synthetic triglycerides (Table 1).* The synthetic triglycerides used in the diets were the saturated compounds trihexanoin (tricaproin), trimyristin, tripalmitin, and tristearin, and the unsaturated compound, triolein.[2, 12]

All mice included in the histological studies were killed by digital compression of the cervical spinal cord. The livers were fixed for at least 48 hours in 10 per cent aqueous formaldehyde. Frozen sections of livers, hearts, kidneys, and abdominal aortas were stained with Sudan black B or with oil red O as previousuly described.[19] Routine paraffin sections of these same organs and of lungs were stained with hematoxylin and eosin.

OBSERVATIONS

The patterns of hepatic liposis in mice fed the 8 diets are summarized in Table 1. An identical pattern of rapid intralobular liposis, beginning centrally, extending peripherally, and rapidly involving the entire lobule, was observed in mice fed diets in which the fat component consisted of lard, butter, C_{14} and C_{16} saturated triglycerides, or C_{18} unsaturated triglyceride (Figs. 1, 3, 5, 7, 8, 9). A less extensive and predominantly peripheral liposis (Figs. 2, 4, 6) was observed in mice fed diet VII (lipid as 100 per cent C_{18} saturated triglyceride) and diet VIII in which 50 per cent of the lipid was C_6 saturated triglyceride plus 50 per cent C_{14} and C_{16} saturated triglycerides. The major portion of the study was devoted to a comparison of hepatic liposis in mice fed natural fats (lard or butter as in diet I) with that in mice fed a diet (VIII) containing 50 per cent (of total fat component) of a

* The synthetic triglycerides were obtained from D. P. I. Division of Eastman Kodak Co., Rochester, N. Y., and from the Hormel Institute, Austin, Minnesota. The suggestions and cooperation of Dr. Walter O. Lundberg, Director of the Hormel Institute, are gratefully acknowledged.

saturated C_6 triglyceride. The groups of mice fed these two diets (I and VIII) were large and the lack of significant changes in the body weights of the members of these two groups during the course of the experiments accentuates their value as material in which two contrasting types of liposis can be studied (Table 2).

Rapid and complete liposis in hepatic lobules. Livers of all mice except those fed diets VII and VIII (Table 1) showed centrolobular liposis within

TABLE 1. PATTERN OF HEPATIC LIPOSIS IN MICE

Fat composition of diets (Each diet contained 40% fat)	*Intralobular pattern of liposis after feeding diet for:*		
	1 day	*3-7 days*	*10-30 days*
I. 100% lard or 100% butter 80 mice	20 mice: central abundant	20 mice: complete lobular	40 mice complete lobular
II. 100% C_{18} Xsat. (triolein) 16 mice	4 mice: central abundant	8 mice: complete lobular	4 mice: complete lobular
III. 100% C_{14} sat. (trimyristin) 18 mice	4 mice: central abundant	8 mice: complete lobular	6 mice: complete lobular
IV. 50% C_{14} sat. *plus* 50% C_{16} sat. (tripalmitin) 18 mice	4 mice: central abundant	8 mice: complete lobular	6 mice: complete lobular
V. 15% C_{14} sat. *plus* 15% C_{16} sat. *plus* 70% C_{18}Xsat. 12 mice	4 mice: central abundant	8 mice: complete lobular	not studied
VI. 32.5% C_{14} sat. *plus* 32.5% C_{16} sat. *plus* 35% C_{18} Xsat. 18 mice	4 mice: central abundant	8 mice: complete lobular	6 mice: complete lobular
VII. 100% C_{18} sat. (tristearin) 19 mice	3 mice: irregular and sparse	8 mice: sparse and predominantly peripheral	8 mice: predominantly peripheral
VIII. 50% C_6 sat. (trihexanoin) *plus* 25% C_{14} sat. *plus* 25% C_{16} sat. 50 mice	6 mice: sparse and predominantly peripheral	14 mice: predominantly peripheral	30 mice: predominantly peripheral

Abbreviations: sat.—saturated; Xsat.—unsaturated.

24 hours after restriction to the high fat diets. The lipid appeared first as small sudanophilic droplets in the cytoplasm of the parenchyma of central zones. During the subsequent two days the liposis spread in a peripheral direction and usually involved all lobular zones at the end of 72 hours

TABLE 2.

Fat composition of diets	*Wt. changes (av.) of mice fed diets for:*		
Diets producing complete lobular liposis within 1-3 days:	*7 days*	*14 days*	*30 days*
I. 100% lard	93 mice −0.3%	100 mice −0.7%	91 mice −0.3%
II. 100% C_{18} Xsat.	8 mice −15%	6 mice −18%	5 mice −24%
III. 100% C_{14} sat.	8 mice −13%	6 mice −16%	5 mice −18%
IV. 50% C_{14} sat. + 50% C_{18} sat.	7 mice −12%	6 mice −13%	4 mice −9 %
V. 15% C_{14} sat. + 15% C_{16} sat. + 70% C_{18} Xsat.	6 mice −2.7%	Not Studied	Not Studied
VI. 32.5% C_{14} sat. + 32.5% C_{16} sat. + 35% C_{18} Xsat.	7 mice −8%	6 mice −16%	3 mice −14%
Diets producing restricted and predominantly peripheral liposis of hepatic lobules during 1-30 days:			
VII. 100% C_{18} sat.	17 mice −8%	8 mice −8%	8 mice −26%
VIII. 50% C_6 sat. + 25% C_{14} sat. + 25% C_{16} sat.	46 mice +2.4%	38 mice +3.5%	30 mice +2.5%

Abbreviations: sat.—saturated; Xsat.—unsaturated.

NOTE: Included in this table are all mice for which body weights were recorded on the 1st day of feeding and on the 7th, 14th, and 30th day subsequently.

(Figs. 1, 8). During the subsequent 27 days of feeding these diets there was a progressive increase of intracytoplasmic lipid (Figs. 3, 5, 7) which was accomplished by formation of large globules or droplets, usually one per cell, as the result of fusion of smaller globules. This process enlarged the cells, and nuclei were often acentric. However, at 30 days cellular enlargement resulting from cytoplasmic accumulation of fat was not sufficient to produce extensive distortion of the basic parenchymal pattern of the liver. The least amount of fat was in the parenchyma of peripheral zones. There

was no specific central-peripheral intralobular gradient in the quantity of lipid in zonal cells, since mid-zonal cells often contained more stainable lipid than central cells. After three days lipid was abundant in both central and mid-zonal cells and was easily demonstrable in cells of the peripheral zones.

Incomplete and predominantly peripheral liposis in hepatic lobules. Complete and abundant lobular liposis did not occur in mice fed diet VII (fat as 100 per cent C_{18} saturated triglyceride) or diet VIII (50 per cent of fat as C_6 saturated triglyceride). Only in the latter group (diet VIII) was the number of mice sufficient to permit a chronologic view of the pattern of liposis. In these mice centrolobular liposis was restricted to very small globules of sudanophilic material and there was no progressive increase in the amount of lipid within the cytoplasm of the parenchyma of this zone. During 3-30 days of feeding there was an accumulation of fat in the parenchymal cytoplasm of peripheral zones of lobules (Figs. 2, 4, 6). The intracytoplasmic liposis in peripheral zones was extensive and in relation to the cytoplasmic enlargement and distortion of individual cells it approached an immediately "precystic" stage (Fig. 4) on the basis of earlier descriptions of hepatic liposis in rats[9] and mice[8, 19] fed hypolipotrophic diets.

In all of the mice used in this study the hepatic reactions to the high fat-choline-deficient diets had not reached the stages in which there is formation of ceroid pigment or changes in the stroma.[19]

Changes in other organs. Hearts, kidneys, and aortas showed no evidence of deposition of fat or other changes from normal. A majority of lungs showed small amounts of diffuse pneumonia. No renal lesions and a very low incidence of myocardial lesions were observed previously in mice fed high fat-choline-deficient diets for as long as 10 months.[19]

Changes in body weight (Table 2). The two large groups of mice fed diets (I and VIII) that produced contrasting types of liposis showed a satisfactory maintenance of body weight. The slight average loss of weight in mice fed lard and the small increase in those fed the C_6 compound do not seem significant (Table 2). The data derived from the relatively large group of mice fed lard as the dietary fat (diet I) and the small groups fed the other diets (II-VII in Tables 1 and 2) show that complete lobular liposis occurs in mice who maintain their initial body weight for 30 days and also in those that lose as much as 24 per cent (diet II) during the same period. Similarly, there was restricted liposis in mice maintaining initial weight (diet VIII) and also in those losing 26 per cent of weight during 30 days of feeding. The loss of weight in mice fed the two (saturated and unsaturated) C_{18} triglycerides (Table 2) gives reason to doubt effective absorption and/or utilization of these compounds in choline-deficient mice.[21]

DISCUSSION

Within hepatic lobules a liposis that was restricted in amount and peripheral (zonal) in location was limited to mice fed diets in which the lipid component was a C_{18} saturated triglyceride or predominantly a C_6 saturated triglyceride. The other synthetic compounds produced a liposis identical with that observed in mice fed lard or butter as the dietary fat. The major lipid components of lard are a 26 per cent level of C_{16} saturated compound and 62 per cent of C_{18} unsaturated; of butter, 11 per cent of $C_{4\text{-}12}$ saturated, 40 per cent of C_{14} and C_{16} saturated, and 36 per cent C_{18} unsaturated.[2, 13]

The study reported here permits no differentiation between deposition of fat supplied by the diet from that synthesized *in vivo*.[8, 17, 21] The present data are in agreement with the results of quantitative analyses[4, 5, 8, 21] demonstrating that maximal hepatic liposis occurs when the dietary lipid of a choline-deficient diet is chiefly composed of saturated C_{14}-C_{16} compounds fed as fatty acids, ethyl esters, or as natural fats. The same analyses demonstrated a decrease in hepatic lipid when C_4-C_{12} or C_{18} saturated compounds were fed. In contrast to the present findings an earlier study showed decreased (from maximal levels obtained with saturated C_{14}-C_{16} lipids) amounts of liver fat when unsaturated compounds including oleic acid (C_{18}) were fed.[4]

The purpose of this study was production of maximal hepatic liposis and no attention was directed to the amount of choline needed to prevent liposis when various synthetic triglycerides were substituted for natural fats such as lard or butter. Supplementation with choline chloride at a level of 0.5 per cent to 0.8 per cent prevents hepatic liposis in rats and mice fed lard containing diets, but not when some of the synthetic triglycerides used here are the dietary lipid.[19, 23]

An initial and immediate centrolobular liposis is a frequent response of hepatic parenchyma of several species including mice to a variety of insults including the actions of choline-deficient diets,[19] starvation,[24] polyhalogens[22, 24] and cortisone.[25]

The patterns of liposis and of cirrhosis in mice[8, 19] and rats[11, 14] fed hypolipotropic diet have received considerable attention. In general it has been concluded that liposis begins centrally[14, 19] and initial cirrhosis is not portal in location.[1, 11, 19] In animals fed such diets the stromal changes that have been described as cirrhosis or reticulinosis are not the direct result of liposis, but are due in considerable measure to the amount and pattern of nodular parenchymal hyperplasia.[11, 14] Rapid and complete lobular liposis, but without a specific central-peripheral pattern of spread within lobules, was ob-

Explanation of Figures

All figures show frozen sections of livers stained with oil red O or Sudan black to show fat.

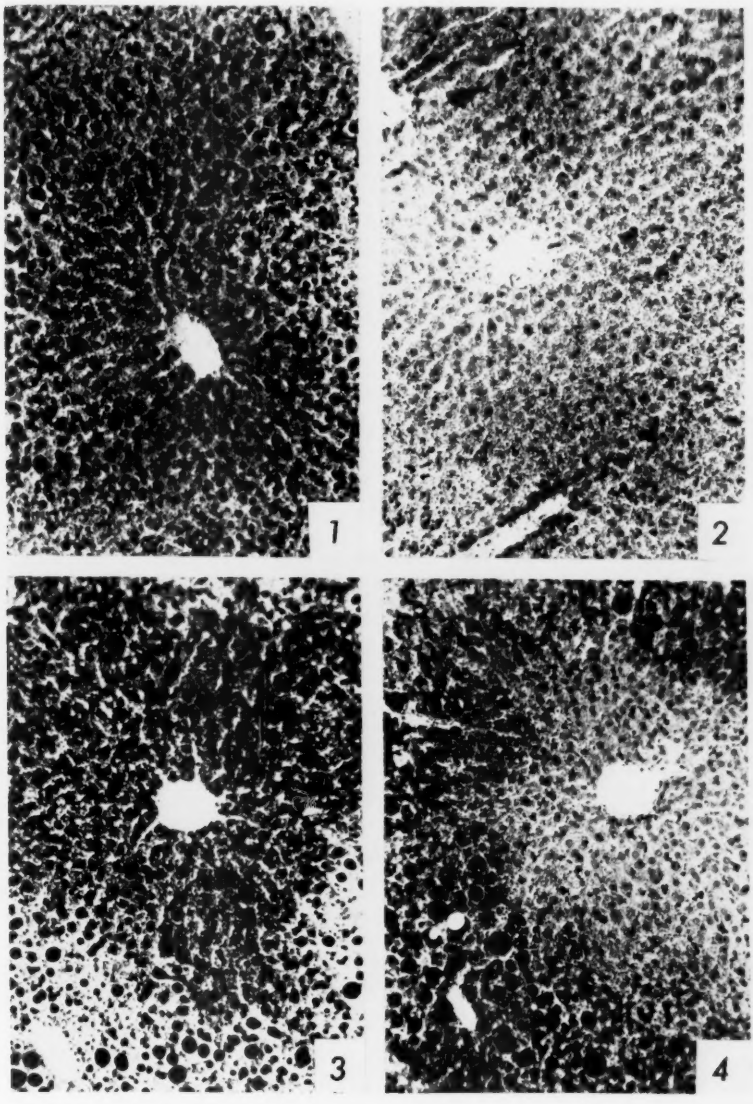

Plate 1

All sections are stained with oil red O, counterstained with hematoxylin; and show central vein.

Figs. 1 and 3. Abundant liposis in mice fed lard containing diet for 3 (Fig. 1) and for 30 days (Fig. 3). x100.

Figs. 2 and 4. Restricted liposis after feeding diet VIII (C_8 saturated triglyceride as 50% of total fat) for 3 days (Fig. 4). Observe the prominent and predominant peripheral (portal) liposis in Fig. 4. x100.

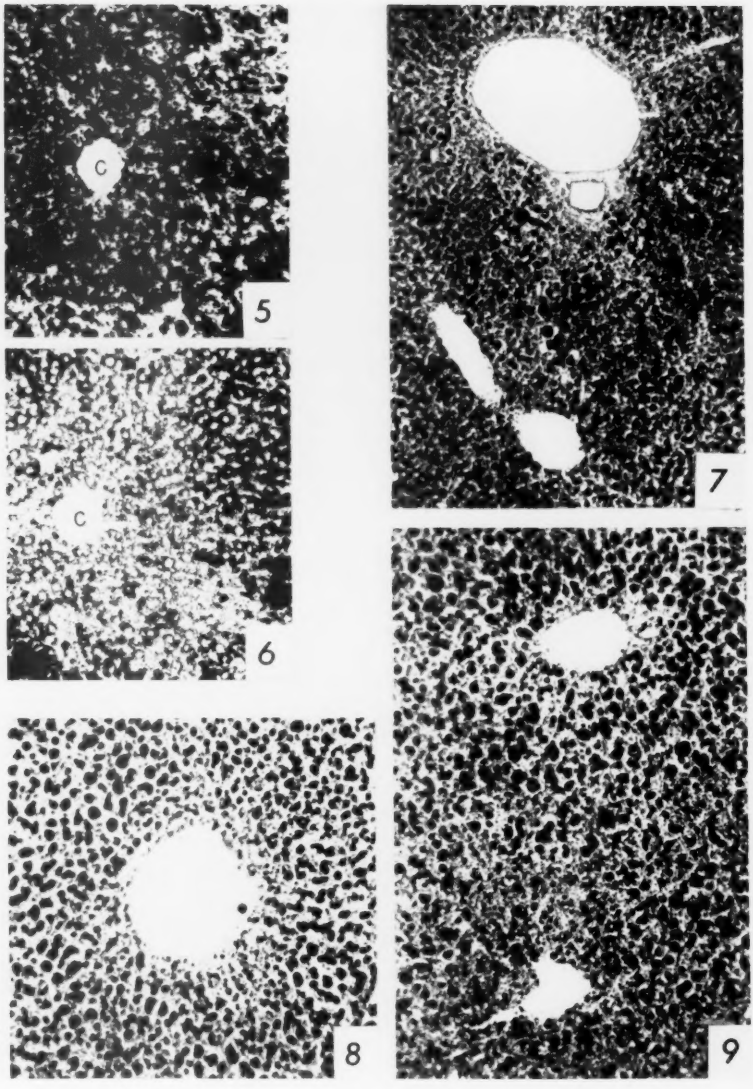

PLATE 2

FIGS. 5 and 6. Complete lobular liposis after feeding diet I (lard) for 30 days (Fig. 5) and a predominantly peripheral liposis in mouse fed diet VIII (50% C_8 saturated triglyceride) for 30 days. "C" indicates central vein. Sudan black. x80.

FIG. 7. Lard diet fed for 3 days. Portal vein, hepatic artery and bile duct are shown in upper one-third of photograph. The extent of liposis throughout the entire lobule is shown. Oil red O. x50.

FIG. 8. Diet V (15% C_{14} and 15% C_{16} saturated plus 70% C_{18} unsaturated triglycerides) fed for 3 days. All portions of lobules were filled with fat in this liver of which the central portion of a lobule is shown. Oil red O stain. x100.

FIG. 9. Diet II (100% of fat as C_{18} unsaturated triglyceride) fed for 7 days. The central vein is in lower portion of photograph, the portal vein in the upper. Lobular liposis is complete, but is more obviously unilocular in peripheral portion of this lobule. Oil red O stain. x100.

served in mice fed choline-deficient diets in which soya flour was the source of protein.[8]

A choline-deficient diet containing C_4 saturated compound (tributyrin) as the lipid component did not produce a typical centrolobular liposis in young rats, but a peripheral liposis was not described.[23] A predominantly peripheral lobular liposis was observed in rats fed a choline-deficient and moderately high (15 per cent) fat diet in which the protein sources were a 76 per cent level of corn meal plus a 3 per cent level of casein.[20] In rats[15] and in mice[19] fed the basic diet (containing lard as fat) used here the initial liposis was centrolobular. The present study was terminated 4 to 6 months before stromal changes occur in livers of mice fed choline-deficient diets.[19]

An initial and continuing peripheral liposis was observed here in mice fed two of the diets and a similar pattern has been described previously in rats[20] fed choline-deficient diets containing corn meal as the major source of protein. This type of liposis does not substantiate the explanation that the fairly broad susceptibility of centrolobular cells to a variety of injurious agents and conditions is due to a regional deficiency of essential blood-borne elements in central zones because of prior utilization of such materials in the more peripheral portions of lobules.[7, 13, 18, 24] It seems clearly demonstrated that both the quantity[4, 5, 6, 21] and the intralobular pattern of hepatic liposis are controlled to a considerable degree by the chemical composition of the lipid components of choline-deficient diets. A better understanding of the liposis awaits demonstration of the separate and combined contributions of the three atypical components to the process. These components are an inadequate level of choline, a high level of dietary fat,[21] and the low level of protein.[14, 15, 16]

SUMMARY

1. An initial centrolobular liposis that involved all hepatic lobular zones within 72 hours was observed in mice fed choline-deficient diets containing butter, lard, C_{14} or C_{16} saturated triglyceride, or a C_{18} unsaturated triglyceride as the lipid component.

2. When the dietary fat was a C_{18} saturated triglyceride or predominantly (50 per cent of total dietary fat) a C_6 saturated triglyceride, the liposis was restricted chiefly to peripheral (portal) zones of lobules and had not extended throughout all lobular zones after 30 days of feeding.

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EXPERIMENTAL ACUTE MONOLOBAR PULMONARY HYPERTENSION†

Necrosis, fibrinoid changes, and cellular infiltrations of pulmonary arterioles have been observed, among other alterations, in extreme pulmonary arterial hypertension. Whether these lesions are the cause or the effect of the high pressure, whether they are the consequence of spasm, or whether they are produced by some other mechanism such as hypersensitivity, remains to be established. Certainly there is a striking resemblance to the vasculitis seen in the lungs in periarteritis nodosa,[28] Wegener's granulomatosis,[15] and the angiitis and granulomatosis described by Fienberg,[14] all of which may be of allergic pathogenesis. It is, however, the very diversity of the conditions in which necrotizing lesions of pulmonary arterioles have been found—various types of congenital heart disease with left to right shunt,[4, 29] functionally common ventricle, severe chronic mitral stenosis,[23, 31] "primary" pulmonary hypertension[18, 22]—which suggests that these changes in the vessels are associated with, or perhaps even produced by, the hypertension per se.

Historically, this concept had a gradual evolution. Although pulmonary vascular lesions were noted in parenchymal disease of the lungs more than 120 years ago,[2] it was not until 1907 that Mönckeberg[25] described "primary arteriolar sclerosis" of the lungs. The tendency was at first to consider the vascular lesions to be a cause of *cor pulmonale*. The idea that, on the contrary, pulmonary hypertension could be responsible for the vascular lesions was first advanced by Moschcowitz[26] in 1927. This suggestion had been previously made with regard to systemic hypertension. A possible sequence of events might then be: Arterial spasm, hypertension, ultimate damage to vessels in consequence of either, or both, of the preceding.

Byrom and Dodson[5] demonstrated the occurrence of "fibrinoid necrosis" in the renal vessels of rats subjected to sudden brief rises of arterial pressure in

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the range of 80-90 mm. Hg by intra-aortic injection of Ringer's solution. Necrosis, and fibrinoid and other exudative changes have been induced experimentally by the administration of adrenalin.[28] The prior administration of dibenamine was noted to prevent both hypertension and lesions. These experiments leave unanswered the question whether vasospasm, or the hypertension itself, is the injurious factor.

In the attempt to produce pulmonary hypertension experimentally, three approaches have been used: (i) Anastomosis of pulmonary to systemic vessels; (ii) resection of pulmonary tissue in order to restrict the output of the right ventricle to a reduced pulmonary arterial bed; (iii) embolization of the pulmonary vascular tree.

Anastomosis of pulmonary to systemic vessels. Levy and Blalock[24] were among the first to investigate the effects of anastomosing the left subclavian artery to the distal end of the left pulmonary artery. In the 12 dogs of their series, pressures just beyond the anastomosis were only slightly elevated, and the vessels of the lung were unchanged when studied histologically six months after the operation.

A similar principle has been employed more recently by several groups of workers. Muller, Dammann, and Head[27] observed medial hypertrophy and zones of intimal proliferation when the distal left pulmonary artery was anastomosed to the aorta, or when the left upper lobe pulmonary vein had been connected surgically with the left subclavian artery a year previously. The extent of the lesion was thought to be correlated directly with the size of the anastomosis. Within the first week, vascular engorgement, intra-alveolar hemorrhage, and red blood cells in the walls of the vessels with a surrounding polymorphonuclear reaction were described. At 11 weeks the vessels had become thickened as a result of medial hypertrophy and intimal proliferation and collagenization.[10] The observations of Ferguson *et al.*[12] were similar, after anastomosis of the distal left pulmonary artery to a systemic artery. Upon re-anastomosing the left pulmonary artery to its proximal stump following a period of hypertension for the previous 6 to 8 months, the lesions were interpreted to regress slowly over the subsequent 25 months.[13] Both of these groups concluded that it was the mechanical influence of the increased blood pressure that caused the vascular lesions.

Restriction of outflow. In dogs permitted to survive after resection of large portions of lung tissue, systolic pressures have become elevated, but mean pressures have not reached levels observed in man with "primary pulmonary hypertension," nor with congenital heart disease accompanied by large left to right shunts, where necrosis and other changes in pulmonary arterioles have been found. The mortality following resection of all but the left upper lobe for

example[2, 6] was high, but in the few survivors the pulmonary arterial pressures immediately after operation averaged 64/8. Postoperatively, there was a tendency for these pressures to fall somewhat. It is noteworthy that survival is more likely in animals in whom lung tissue has previously been caused to distend by resecting the opposite lung at an earlier stage.[7] This suggests an accommodative increase in size of vessels upon distention of lung within certain limits. Overdistention of residual lung tissue after resection of other lobes is undesirable, however.

Embolization of pulmonary vascular bed. The importance of the organization of thrombi in the genesis of "arteriosclerosis" or "endarteritis" in pulmonary arteries has been stressed. It has been suggested that "primary" pulmonary hypertension may be in fact secondary to clinically inapparent embolization with subsequent organization. C. V. Harrison[16] injected finely comminuted blood clots into the ear veins of rabbits and produced "arteriosclerotic" changes in the pulmonary artery, which were interpreted to be simply the result of organization of the clots. These views have been supported also by Barnard,[3] and by Thomas, O'Neal, and Lee,[32] on the basis both of experimental work and studies of human tissues. In these observations the existence of hypertension was merely inferred from the fact of right ventricular hypertrophy. Actual measurements were made by Jaques and Hyman[20] of pressures in the right ventricle after embolization. Transient hypertension was observed which was associated with apnea, and bradycardia, as in the Jarisch-Bezold syndrome, suggesting the operation of reflex mechanisms. They postulated that repeated rapid rises in pulmonary arterial pressure might be responsible for the necrotizing and exudative vascular lesions which they found at necropsy. Fibrinoid necrosis was absent in these animals. Since the injected material was not sterile, it may well be asked whether some of the changes might not have been of bacterial causation. Severe persistent hypertension was not observed in these animals.

The present experiments were designed to test whether an acute rise in pulmonary arterial pressure approaching systemic levels over short intervals of time could in itself result in damage to the pulmonary artery.

MATERIALS AND METHODS

The pulmonary vasculature of the left lower lobe in adult dogs was isolated into an almost closed chamber by cannulating the lobar artery and obstructing the draining pulmonary veins. Pressure in this system was increased by forcing heparinized blood or 0.9 per cent saline solution at the necessary rate into the cannula. Blood was withdrawn from the systemic circulation to accommodate for the volume introduced into the pulmonary artery with each episode of the artifically induced hypertension.

Infusion cannula. The infusion cannula was designed (a) to make possible the introduction of fluid under pressure without imparting significant frictional resistance of its own, and (b) to permit the measurement of pressure at the end of the cannula. It was a double-lumened tube of polyethylene (Fig. 1). The outer tube measured 3.76 mm. in internal diameter (PE 360) and was attached at one end to a glass T-tube, the opposite end of which served for introducing fluid. Smaller polyethylene tubing

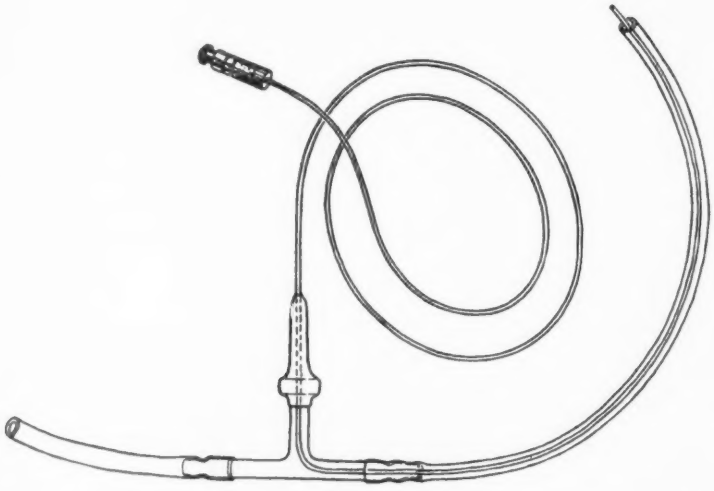

FIG. 1. Infusion cannula. The large outer polyethylene tube at the right is introduced into the vessel. The inner tube is used to measure pressure. Its end projects farther into the vessel than that of the outer tube. Fluid is introduced through the tube at the left from the infusion apparatus (Fig. 2).

1.14 mm. internal diameter (PE 160) was introduced into the lumen of the outer tube through the vertical limb of the "T" to which it was sealed watertight. The inner tube, used for measuring the pressure in the left lower lobe pulmonary artery, was made to project 3-4 mm. beyond the end of the infusion cannula.

Infusion apparatus. The pressure infusion apparatus (Fig. 2) consisted essentially of two graduated chambers, a pressure bottle, and a suction bottle. The pressure bottle itself (No. 3), was connected to an air pressure line. The latter was provided with a bypass, at "C," for instantaneous release of pressure, and with a screw clamp pressure control for fine adjustment. The pressure bottle was surrounded by a water jacket in order to maintain the infusate at body temperature. The "T"-tube in the exit line to the infusion cannula was connected to a 100 cc. syringe through a side arm by means of which peaks of pressure could be suddenly applied to the pulmonary artery.

The suction bottle (No. 2) was used to remove blood from the animal in a volume equal to that which was infused. It was connected to a suction line via a safety trap

(No. 1). The blood was obtained at the appropriate time and rate from a femoral artery cannulated for the purpose. The suction bypass at "A," furnished a method for instantaneously releasing suction. Between infusions blood was admitted from the suction bottle to the pressure chamber by unclamping, at "B," the short length of tubing that connected them.

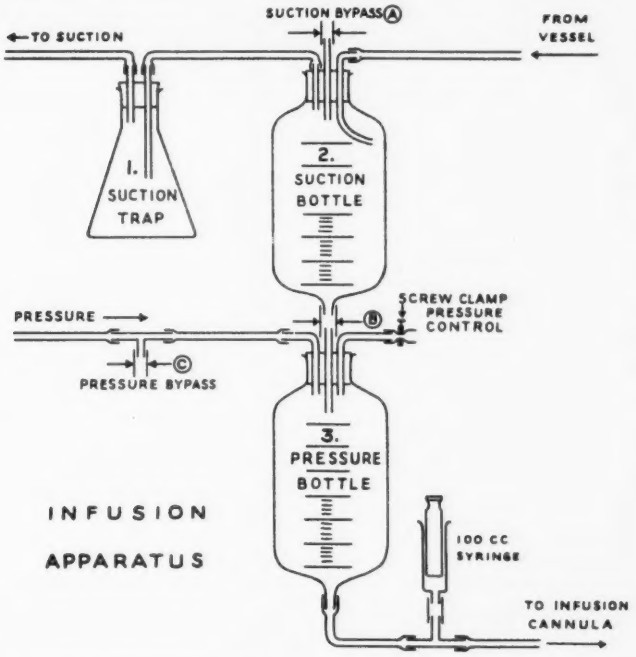

FIG. 2. Infusion apparatus as used for introduction of blood into the vessels of the left lung. Detailed explanation in text under "Materials and Methods."

The entire infusion apparatus was sterilized before use with "Zephiran" (Benzalkonium chloride) 1:1,000, and thoroughly washed with sterile 0.9 per cent saline before use.

Snares. In order to occlude the left lower lobe pulmonary vein repeatedly but without trauma, special snares were devised (Fig. 3). Three flanges were created on a segment of polyethylene tubing and holes were placed in line on two sides to serve as a guide for thin polyethylene tubing, or 2 mm. wide umbilical tape. "Zephiran" was used to sterilize the snares.

Perfusant. An initial volume of several hundred cc. of blood was obtained aseptically from a cross-matched heparinized (1½ mgm./kg.) donor for the purpose of priming the infusion apparatus. The donors were given 600,000 units of aqueous penicillin, and

200 mgm. of streptomycin on the day previous to, and also on the day of the exsanguination.

In one group of animals sterile 0.9 per cent saline was used as the perfusant.

Experimental procedure. The animals were fasted for 18 hours before the experiment. Just prior to the operation penicillin and streptomycin were administered intramuscularly as for the donors. Anesthesia was with pentobarbital sodium (30 mgm./kg.) intravenously. Polyethylene cannulas were placed in a femoral vein and in both femoral arteries. The former was used for delivering heparin and the anesthetic solution as needed. One of the arterial cannulas was used for recording systemic pressure with a Sanborn electromanometer. The other was connected to the suction bottle (No. 2 in Fig. 2) in order to keep the total blood volume of the animal in balance, when the volume necessary to secure the desired pressure rise in the lobar pulmonary artery had been infused. Positive pressure breathing was maintained with the aid of a Burns demand pneumophore interposed between an oxygen tank and the intratracheal tube.

Access to the left pulmonary artery was obtained by an incision through the fifth left intercostal space. Snares were placed about the left main pulmonary artery, the left lower lobe pulmonary artery, and the single large pulmonary vein that drains this lobe. The blood flow was not impeded until the appropriate moment in the experiment. The left upper lobe pulmonary artery was tied distally and transected while the left main pulmonary trunk was occluded by the snare. The infusion cannula was inserted via the transected proximal stump of the left upper lobe pulmonary artery into the lower lobe trunk, with the end of the outer element just distal to the snare that had previously been placed about that vessel. It was then secured with an F silk tie and the outer tube, which had previously been filled with perfusant, was attached to the infusion apparatus, and the inner element was connected to the Sanborn pressure recorder. In some animals pressures were also recorded with additional channels of this apparatus from the main pulmonary artery and left atrium by passing fine polyethylene catheters into these structures. Immediately after cannulation the animals were given heparin intravenously in a dose of 1.5 mgm./kg.

The pressures were raised in the cannulated left lower pulmonary artery after pulling tight the snares surrounding the cannula and the main left lower lobe pulmonary vein. Fluid was rapidly infused until the required pressure was attained, and the desired oscillations were produced with the aid of the syringe connected in the exit line of the infusion apparatus. The total infusion times were obtained by increments of one minute, with one- to five-minute rest periods between infusion.

When hypertension had been maintained for the planned interval of time, the cannula was withdrawn, the stump of the left upper lobe pulmonary artery, by way of which it had been inserted, was tied, and the snares were removed. A loose loop of silk, however, was left in place of the snare about the vein to establish at the subsequent post-mortem examination whether the principal drainage of the lobe had in fact been occluded. The chest was closed in layers, negative intrapleural pressure was restored, and the animals were permitted to recover. They were maintained on daily injections of penicillin and streptomycin in the same dosage as during the preoperative period.

The animals were divided into three groups as follows:

Group I. Ten minutes of hypertension induced by infusion of blood into the isolated vasculature of the left lower lobe, with pressures oscillating between 50 and 100 mm. Hg, and with 20-60 oscillations in that range per minute.

FIG. 3. The snare as used for occluding the left lower lobe pulmonary vein. This yoke-like arrangement for the soft polyethylene tube, or umbilical tape, prevents injury to the vein by crushing or undue torsion.

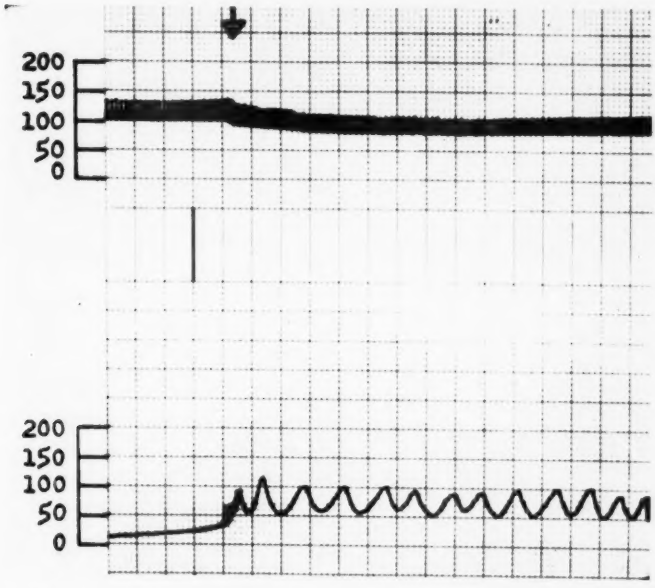

FIG. 4. Pressure changes during infusion with blood. Infusion pressure along bottom tracing. Systemic arterial pressure at top. As the infusion pressure is maintained between 50 and 100 ml. Hg, there is a drop in the systemic arterial pressure and, after an initial tachycardia, a slowing of the heart. Each space as delineated by the solid upright lines represents approximately two seconds.

Group II. As with group I, but with a total of 20 minutes of hypertension.

Group III. Hypertension induced by infusion of sterile 0.9 per cent saline with pressures oscillating between 100 and 150 mm. Hg for a total of ten minutes but otherwise as in group I.

Animals of each group were sacrificed at 3, 5, and 7 days after the induced hypertension by an overdose of sodium pentobarbital, and the necropsy was performed at once. In each instance smears and cultures were made of tissue obtained aseptically from the left lower lobe. The smears were stained with the Gram stain. Cultures were made on blood agar plates and on brain-heart infusion, and thioglycollate broths. Blocks of the left lower lobe were prepared for comparison with tissue from the right lung. The fixative was 10 per cent neutral formalin. Sections were stained with hematoxylin and eosin, and, after mordanting with $HgCl_2$, with Lendrum's picro-Mallory, a method especially useful for the demonstration of fibrinoid material.

OBSERVATIONS

Observations during acute pulmonary hypertension. To bring the pressure in the left lower lobe pulmonary artery to the desired level it was necessary to infuse several hundred ml. of blood at a rapid rate. At a certain point, infusion of very little additional fluid was sufficient to raise the pressure suddenly. Pressure peaks could then be maintained by small increments of perfusant from the syringe that had been attached for the purpose to the infusion line (Fig. 4).

Only in one animal was it established that a small pulmonary vein from the left lower lobe had not been ensnared during the infusion. This animal required more than the usual infusion to attain the desired pressure. In the others, venous egress of blood from the lobe could take place only by way of bronchial venules along the major bronchi. These are notably small in the dog.[19] These observations, then, bespeak the enormous distensibility of the vasculature of the lung. Other evidence for this has been established by the observation that as much as a three to fourfold increase in right cardiac output above the normal resting level can be accommodated without rise in pulmonary arterial pressure.[9, 17, 29] The same is indicated after resection of pulmonary tissue or ligation of vessels as described above.

In animals of group III where the infusion pressure oscillated between 100 and 150 mm. of mercury, considerable leakage of the saline perfusant into the air spaces took place. Thus in one dog, approximately one-fourth of the volume of saline infused into the left lower lobe pulmonary artery was recovered from the trachea. When heparinized blood was infused, in animals of group I and II, there was evidence also of intra-alveolar hemorrhage, but this was remarkably slight.

The gross appearance of the lung during infusion was remarkable. It became enlarged, tense, and firm, like erectile tissue. The gross appearance

was not unlike that of liver, but the color was a brilliant rather than a cyanotic red. Blood used for the infusion was fully oxygenated.

Developmental experiments. Five of the 21 animals died during the operation or shortly thereafter, chiefly during the time when the infusion apparatus, cannulas, and techniques were being developed into their final form. Most of these animals suffered from excessive rupture of the capillaries with accumulations of blood and fluid in the infused lobe. Another expired several days postoperatively from bilateral pneumonitis. This was the only animal in the entire series of 21 who developed an obvious pneumonia.

Four other animals studied during the developmental period survived for the designated interval postoperatively (5-7 days) and were then sacrificed. The general plan of the experiment was that of animals in group I, but various types of cannulas were used for infusion, and there were other deviations in procedure from that which was finally adopted. They were therefore not included in the final groupings despite the fact the results were not essentially different.

Observations on animals of groups I and II. It appeared to make little difference whether the pulmonary artery pressure was maintained for approximately 10 or for the 20 minutes, at between 50 and 100 mm. Hg peak pressure. There were four animals in each group. One dog in each group was sacrificed at three, two at five, and one at seven days postoperatively. It is remarkable that even at three days, the lobe that had been subjected to the hypertension deviated little from the others grossly, in contrast to its appearance immediately after operation. It was now pink but slightly firmer and less crepitant than the remainder of the lung tissue. Several of the dogs had small foci of atelectasis. The left pleural cavity had merely fibrinous adhesions and did not contain an excess of fluid in most of these animals. In two there were 20 and 100 cc. respectively of clear serous fluid on the left side. In another a few adherent clots were present. The main left lower lobe pulmonary artery and its grossly visible branches were in all instances patent. Microscopically, in three lungs of group I and in one of group II, thrombi were found in some of the smaller pulmonary arterioles. These might possibly not have originated *in situ* but have come as emboli from the more proximal parts of the left pulmonary artery that had been subjected to transection and cannulation. The walls of these arterioles did not show fibrinoid changes, necrosis, or significant cellular infiltrations. There was residual interstitial edema of the connective tissue of the lung and remarkably little intra-alveolar hemorrhage. Some of the red blood cells had been phagocytosed, and hemosiderin had already been formed, especially after

the fifth day. There was no actual necrosis of parenchyma to suggest infarction.

Direct smears of the left lower lobe for bacteria were negative in every instance, and in three of these animals the lungs were also sterile upon culture. In the other dogs of groups I and II, the cultures yielded in four a Gram-positive bacillus, and in one a Gram-negative bacillus. These findings are not surprising, since it is well known that the blood of dogs frequently contains these organisms in the absence of known disease. There was no evidence of a bacterial pneumonitis in animals of these two groups.

Observations on animals of group III. In two animals of group III the pressure had been made to oscillate between 100 and 150 mm. Hg for a total of 10 minutes, but in the third it was not possible to reach pressure peaks in excess of 100 mm. of mercury, since the saline rapidly entered the respiratory passages. Despite this indication of ruptured vessels, the animal survived in apparent good health for seven days after the infusion. The gross and microscopic appearances were similar to those of animals in groups I and II. Only one of the three had thrombi in small pulmonary arteries. Smears were again negative in all instances as were cultures in one dog, but in one a Gram-positive rod was cultured and in a third a pseudomonas grew in the thioglycollate broth.

Reflex phenomena. Although considerable dissection was required to accomplish the cannulation without excessive loss of blood, some of the animals exhibited slight systemic hypotension and bradycardia when the pressure in the left lower lobe pulmonary artery was elevated (Fig. 4). This confirms observations of Churchill and Cope,[8] and of Downing,[11] but in the more restricted field of a single lobe, rather than in the entire lung. A systematic and detailed study of reflex phenomena was not made in animals of the present series. Robicsek[30] found evidence of general pulmonary vasospasm when pressure in a single vein of an isolated lobe was raised by retrograde infusion.

DISCUSSION

It was thought that an analysis of various factors that could lead to necrotizing and exudative changes in the pulmonary arteries could best be begun simply by raising the intravascular pressures in the lung. Allergenic materials could be avoided by the use of saline solutions or blood of the same animal. Furthermore, anoxia could be prevented by the use of fully saturated oxygenated blood as the perfusant and maintaining ventilation in the lung. Actually, however, there is evidence that raising the pressure in pulmonary

vessels is not a simple mechanical process, but that it may be accompanied by reflex arteriolar spasm in the lung itself. This is suggested for example, by observations of Wood, Besterman, Towers, and McIlroy.[34] They found that acetylcholine would reduce pulmonary vascular resistance in instances of severe mitral stenosis, thus demonstrating that the hypertension was associated with the presence of precapillary factors, as well as with the postcapillary barrier of the stenotic valve.

The absence of significant changes in the pulmonary arteries when these were subjected to sharp sudden elevations in pressure may be interpreted in several ways. Firstly, one may question whether the pressure rise in the arterioles was as high as was recorded. The design of the cannula as finally employed is such that the pressure is obtained at a point well beyond the end of the infusion cannula. Moreover, flow had practically ceased when the desired pressure level had been attained, and thus it is reasonable to conclude that pressure in the proximal segment of the left lower lobe pulmonary artery reflected that in the smaller arteries. Rupture of vessels with intra-alveolar hemorrhage was further evidence that the smaller vessels were subjected to a high pressure. Pressure in the ensnared venous segment draining the lobe was, however, not measured.

Secondly, it may be that although the pressure to which the pulmonary vessels were subjected was certainly of a magnitude associated with severe vascular changes when chronically maintained, the duration of the hypertension was insufficient. It is doubtful that the experiments could have been prolonged beyond the total of 20 minutes of hypertension at levels of 100 mm. Hg or above, despite the "rest periods," without producing massive intra-alveolar hemorrhage. If, as has been suggested by Lendrum,[28] fibrinogen is forced into the walls of vessels exposed to excessively high intraluminal pressure, it might be expected that 20 minutes would suffice for the process. The dogs of Waters and de Suto-Nagy's[33] experiments were subjected to hypertension over periods of approximately one hour. The experiments of Byrom and Dodson which resulted in the production of arteriolar lesions were of even briefer duration.

A third possibility is that it is not the pressure rise but rather spasm that damages the vessels, and that the dissection necesary to introduce the cannula had damaged nerve fiber, that may be concerned in evoking the spasm. This is not to be denied, inasmuch as pressure in the main pulmonary artery was not altered. Reflex activity involving pathways from the lung to other structures was demonstrated, however, in some of the animals who exhibited bradycardia and systemic arterial hypotension when the pulmonary arterial pressure was elevated.

Fourthly, it may be that hypertrophy of muscle such as that which has been found in chronic pulmonary arterial hypertension, is necessary before acute vascular changes will result from sudden high peaks of pressure. This seems unlikely, since acute lesions have been observed in pulmonary arterioles as large as 450 microns in cross-sectional diameter which are normally endowed with smooth muscle.[28] Nevertheless, the suggested possibility remains to be investigated.

SUMMARY AND CONCLUSIONS

The effects on the pulmonary vasculature of acute pulmonary hypertension were investigated in the previously normal dog. When pulmonary arterial pressures in a single lobe were repeatedly oscillated between 50 and 100 mm. of Hg by infusions of blood for a total period of as long as 20 minutes while the draining veins were temporarily occluded, or between 100 and 150 mm. of mercury by infusions of saline for a total of 10 minutes, no vascular lesions except thromboses of occasional small vessels were observed. High pressure when maintained over these short intervals does not in itself produce passage of fibrinogen into the wall. The lungs recover remarkably well from the intense congestion observed during the period of hypertension, with little residual hemorrhage.

The observed absence of vascular lesions may be because the hypertension is of insufficient duration, or because spasm of pulmonary arterioles has not occurred as a result of interference with nervous pathways, or finally, because a preliminary phase of muscular hypertrophy in the pulmonary arterioles is necessary. Experiments of another design are necessary to test these and other possibilities.

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The Board of Editors

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COMMENTARIES ON MEDICAL EDUCATION AND MEDICAL RESEARCH IN LATIN AMERICA 1958**

A generation ago one might have looked at the field of medicine in Latin America as something interesting but foreign, and, in spite of the Monroe Doctrine, something of little immediate concern to us. It is not so today, not only because we have become more globally minded and more aware of the international implications of medical science, but because through both non-government and government agencies so many American dollars have been poured into Latin America to bolster medical activities that this huge geographical area south of the Rio Grande has become a place where we now have a stake; that is, unless we have become quite indifferent to the way our government spends money in foreign areas.

Obviously this interest in improving medical education and public health is not new nor has the support come from Federal agencies alone. The Rockefeller Foundation was the pioneer and has continued in this field the longest[1]; the Kellogg Foundation[2] has also been active. The Federal agencies which are interested in the extension of medical education, medical research, and public health facilities in Pan America include: (i) the P.A.H.O., or Pan American Health Organization which is the American branch of the World Health Organization, known until recently as the Pan American Sanitary Bureau[3]; (ii) the Medical Division of the International Co-operation Administration (I.C.A.), which represents the U.S. State Department[4] and is now collaborating with the National Academy of Sciences in

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** During September and October 1958, the author visited 11 Latin American countries as senior consultant to the International Fellowship Division of the National Institutes of Health, U.S. Department of Health, Education and Welfare. A variety of medical institutions or agencies, numbering 34 in all, were visited, and discussions were held with more than 100 individuals who represented the fields of medical education, public health, and medical research.

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[3] Report of the Director of the Pan American Sanitary Bureau, Jan. 1954-Dec. 1957, Washington, D. C., 1957, pp. 103-116.

[4] International Cooperation Administration, Monthly Reports of the Public Health Division, 1958.

its visiting Research Scientist Program[5]; and, most recently, the International Fellowship Program of the National Institutes of Health[6] as well as the Research Grants Division of that organization which has been aiding Latin American scientists for some years. The support of these agencies, furthermore, is not limited to the granting of money, for some of them have maintained a sort of medical foreign office in some areas, led by men who are devoting no small part of their lives to this task.

As to historical background, it should be pointed out that it is only within the past generation that U.S. medicine has begun to have much of an impact on the countries of South America. Prior to World War II medical education in all of Latin America seems to have been largely dominated by French influence. Not only was the local medical curriculum modeled after the French system, but hundreds of Latin American medical students and physicians went to Paris each year to matriculate for undergraduate and postgraduate training. Perhaps they still do, but Europe was certainly the mainspring of medical knowledge for the Latin American in the nineteenth century. The language difficulty in France for a person speaking Spanish or Portuguese was not as great as elsewhere, and besides, for South America in particular, Paris was the cultural center of the world. Today, as in former years, one is apt to see in both large and small cities all over Central and South America, many a brightly polished shingle outside a doctor's office, on which his name is inscribed and under it, often in large letters, the information that he is a graduate of the "Faculté de Medicine de Paris." Today, however, there is already evidence of a shift, and it is to the United States that Latin America now turns. By tomorrow we may see shingles beginning to appear advertising a degree from the University of Michigan or Pennsylvania.

Latin American interest in medical education in the United States received considerable impetus after World War II, and after communications with Europe had been interrupted for at least five years. Also, by 1940, U.S. medicine had achieved what amounted to a kind of world-wide recognition which it had not had before. The shift was not without its "pressure groups" partially responsible for this change in allegiance. The probabilities are that the Rockefeller Foundation was very influential here: through the example set by the fine and effective work in its local research laboratories on yellow fever and other subjects; through its international fellowship program based on the careful selection of candidates and the placement of the fellow on his

[5] Visiting Research Scientist Program. Article in *Science*, 1959, *129*, 28.
[6] In Hearings before the Subcommittee on Appropriations, House of Representatives, 85th Congress, 2nd Session, Washington, D. C., 1958, pp. 355-356.

return to his native country, and through the discriminating kind of support this Foundation rendered to Latin American medical schools both in the matter of teaching and grants-in-aid for research programs. These are examples of what could be called effective missionary work in the broad field of medicine. It was an example soon copied and expanded by other agencies both private and governmental.

In considering the impact which U.S. medicine has had on Latin America and its potentialities, it is worth speculating for a moment on the importance which matters pertaining to health now hold in the field of international relations. The discovery has been made that health is a focal point on which there can be universal agreement among nations even in the face of much disagreement on other matters. Regardless of the ways in which countries and peoples disagree as to types of government, economic policies, religions, and so on, they usually will admit that the elimination and cure of disease is desirable. They may not agree as to which is the best kind of medical philosophy to follow, but, none the less, that the principles of modern medicine, and particularly, preventive medicine are essentially sound is a point on which there is a good chance for unanimity. It thus becomes a field not only deserving a very high priority but one easily exploited, and anyone who attempts to preach the gospel of modern medicine today, on an international basis, faces the dual responsibility of being sure his preachings are correct scientifically—and diplomatically. Actually, it is in this broad field that World Health Organization has attempted to deal with these questions and it is succeeding in designing proper policies.

Thus our efforts to introduce our own medical philosophy into Latin America are naturally involved in matters of local prejudice and of expediency. But we are succeeding slowly, as judged by many yardsticks, not the least of which is the increasing number of Latin Americans who have been trained on fellowships in the United States and who often return to the United States for subsequent training. Such international training will probably shape the course of medical education in the Latin American medical schools in the future. It is not limited to physicians but also has been made available in the form of traineeships for nurses, sanitarians, technical workers, etc. Another example of the growing linkages is that at least one South American school has made special arrangements for a particularly close international association, viz. between the Medical School at Cali, Colombia, and the Tulane University School of Medicine in New Orleans. The arrangement calls for visiting professors on an exchange basis and the continued mutual exchange of ideas which should go far in bringing the two systems of medical education together.

To date, this over-all program might seem to be one which could lead progressively to a better and closer relationship between the United States and many Latin American countries as far as medical affairs are concerned. However, there are plenty of pitfalls and dangers of too rapid expansion. For example, with regard to the fellowship programs, which at present are being expanded, a word of caution is not out of order. International Fellowships, of course, can be of all kinds, including research, training, and travel fellowships, etc. They are easily abused. A good international fellowship experience can do much for mutual understanding, but a bad experience can do just the opposite. If candidates are not carefully picked and carefully sponsored by the host institution in the United States, the ex-fellow can readily spend the rest of his life elaborating on his bad time in the U.S. This would be a so-called example of international understanding in reverse.

MEDICAL SCHOOLS

With few exceptions medical schools in Latin America are controlled and supported by the State. The extent to which such government control is responsible for the designation of faculty members and for the educational policies must vary greatly. However, this "government influence" should be recognized at once, if a critique of Latin American medical schools is to be attempted.

Faculty. As to the calibre of men occupying chairs and directing departments in the Latin American medical schools we visited in 1958, it should be said at the start that there are outstanding figures whom many of the best medical schools in the United States would be proud to number among their faculty. These men are individuals who might have been outstanding almost anywhere, for there seems to be little relationship, as judged by our standards, between their stature and the over-all scientific level of the environment from which they come, or the stature of the medical school which they represent.

Be that as it may, considerable efforts are afoot to raise the standards of certain existing medical schools. One of the transitions being effected in some places is the shift from a part-time to a full-time system for teachers. The latter type of organization, which so far is for the exceptional medical school, calls for this reform both in the preclinical and the clinical years, and it calls for laboratories equipped for use by members of the full-time faculty who have had training and the facilities to carry out research. This is a quite different situation from that which existed almost universally in Latin America until recently—and which existed in the United States

prior to 1920—i.e., the time-honored system under which clinical teaching was done by important practising physicians or surgeons, often men of considerable stature and experience, who dropped in at the medical school for an hour or two a day, or even an hour or two a week, to make rounds or give lectures usually to very large classes. Much of the emphasis on the value of full-time teachers has come about through the influence of the Rockefeller Foundation, which also had no small part in bringing about a change along these lines in U.S. medical education a generation ago. In Latin America the transition to the full-time system of teaching in the *pre-clinical* sciences is well on its way but with the *clinical* teaching it is still in the early stages. Discussions held with physicians and clinical teachers in Latin America in 1958 were reminiscent of those which beset the medical profession in the 1920's in the United States, when the evils of full-time medicine were expounded by experienced physicians who saw little that was good in having "laboratory men" teach the coming generation of students about the art of medicine. In defense of these arguments for the old system one might recall that it took at least a generation in the United States between the time when the full-time system for teaching internal medicine, pediatrics, and surgery was first painfully introduced into clinical departments of a few medical schools, and its final acceptance. And, we might recall that some of the best U.S. schools today have not accepted the principle of a rigid adherence to full-time teaching for their professors of medicine or of surgery, particularly for the surgical specialties.

Medical curricula, standards, and student activities. Today, in 1958, the curriculum in Latin American medical schools still retains much of the European pattern. It usually consists of a six-year course for which the entrance requirements may vary considerably. As a rule, a college degree is not required. By and large, the first two of the six years cover subjects which in the United States would be given in a premedical college course: languages, biology, physics, chemistry, and some physiology. In addition, a course on biostatistics may be included, and there may be others coming under the head of the social sciences, human biology and social anthropology. In some respects, therefore, this increased amount of time available for the teaching of modern medicine would seem to have advantages. For, in the United States today important and timely courses have been denied admittance to the already overcrowded four-year curriculum in the struggle to maintain and expand established disciplines at the expense of the new.

As for entrance requirements and scholastic standards in Latin America, these seem to range all the way from what seem to be no requirements at all to those which would be considered moderately strict by U.S. standards. An

example of what can exist is the situation at the Faculty of Medicine of the University of Buenos Aires. Here the school is still staggering under the powerful influence of the regime of Peron, the recently deposed political dictator. Peron is said to have championed the principle that no young man or woman who desired to study medicine should be denied that privilege and that all aspiring to be students should be given the chance through admission to medical school without the red tape of entrance requirements. Obviously one of the effects of this "liberal policy" is to make the entering class in medical school very large. The actual number of students in the first- and second-year classes was said to be about 2,000 and 1,500 respectively. Furthermore, year after year the first-year class had continued at this size apparently because there was no effective mechanism whereby a student could be dropped from the school because of poor scholastic standing or failure to pass examinations. On the other hand poor marks were sufficient to prevent a man from being promoted to the next class. As a result of this, the fifth- and sixth-year classes were smaller than those of the first two years, being listed as being about 500 in size, still large when it comes to bedside instruction, ward rounds, and individual teaching in the out-patient departments.

Another effect of this liberal attitude towards those who desired to be medical students was that the first two classes had a large quota of men who had become more or less "professional students." Some of them had been matriculated in the medical school for as long as 10 years! Often this had been on a part-time basis while the student worked at some job during most of the day and dropped in for a lecture or two in the evening in the hope that he might learn enough this way to pass examinations which might allow him a promotion. It would be an understatement to say that such students did not contribute to the academic atmosphere of the school. It is among this group of seasoned, professional students, we were told, that one finds the rabble rousers, who play no small part in extracurricular activities of university life in Latin America. Student strikes, vociferous demands for social reforms, and other kinds of reform are frequent enough in Latin America to be regarded as a natural, periodic state of turmoil, from which few institutions are free for long. We encountered our fair share of examples of this during the autumn of 1958. A two-month-long strike, on the part of the whole medical profession, had just terminated in Buenos Aires in mid-September (which corresponds to mid-March in the northern hemisphere), and so their current academic year had been badly disrupted. Medical students had joined in this strike and apparently had had some voice in the negotiations. Indeed, medical students may influence policies governing

their education in some of the South American countries to a surprising degree by having representatives on the Directing Councils of the medical school. In the Faculty of Medicine of the University of Buenos Aires this Council numbered 15, of which 8 were members of the medical faculty, 3 represented the alumni, and 4 the student body.

We were also treated to the opening blasts of a students' strike in Montevideo, Uruguay. The method of attack was the daily installation of a blaring loudspeaker set up in the street in front of the office of the Rector of the University. All day long it re-iterated the students' demands. In Lima there were manifestations in the streets by students of the University of San Marcos, who were following the lead of other Peruvian universities by demanding self-government in the running of the University to a greater degree even than exists in Argentina. Most of the various faculties in Lima, law, arts, etc., had already capitulated to these demands although the medical and dental faculties held out. In Panama City, students were rioting for unknown causes and setting off bombs in the streets to such an extent that it was considered unsafe to venture into the city at all.

Obviously there is nothing unique about political demonstrations of this kind being held by university students either in Latin America or anywhere else, but there they were regarded as a matter of course and without misgivings except by the university faculties. The instances in the autumn of 1958, mentioned above, were to some extent overt demonstrations of general unrest in countries which were currently suffering from a ruinous kind of economic inflation. Under such circumstances students could agitate for reforms better than most. Indeed, the lads from the University of Buenos Aires had been credited by some as actually leading the movement which had ousted the dictator Peron from Argentina, and it was generally agreed elsewhere that other dictators had suffered at the hands of students. Occasionally it was admitted that they went too far, with ill-advised demonstrations. We also were not so impressed by the frequency or the violence of these demonstrations, or student rallies, as we were by the fact that *medical* students should have taken such an active and prolonged part in them.

One other final fact regarding medical students, which may not be at all universal but apparently is common in Brazil, the largest Latin American country, was that a considerable percentage of the graduates in medicine never intend to follow this profession seriously. Instead, having obtained their degrees, many of them go straightway into some other field, very often politics where they are not averse to retaining the title of "Doctor." To some observers this appeared to be a waste of good medical education; to others

the feeling was that a medical education, comparable to an education in law, fitted one quite well for other walks of life.

Public health and preventive medicine. It is on the practice and teaching of preventive medicine that various U.S. agencies are now laying particular stress in Latin America. The aim here has been not only to point out gaps in public health practices, but to bolster existing health organizations and expand them. Certainly the need for extensive disease control programs is still a crying one in Latin America—campaigns for the eradication of malaria, schistosomiasis, enteric infections, Chagas' disease, nutritional deficiencies, and other endemic diseases, mostly, but not all, infectious in nature. The two schools of public health which we visited, one in Sao Paulo, Brazil, and the other in Santiago, Chile, had long been well aware of the situation and were busy with laboratory and field work on these national problems.

As for teaching the concept of preventive medicine or social medicine in the undergraduate medical schools, until a decade ago this had received short shrift. Today in some places things are different and, in this effort, U.S. agencies have had no small part. The aim has been to point out the new responsibilities for the modern physician, and that the day is over when the physician can regard his job as limited solely to diagnosis and therapy of one single episode in an individual patient, in other words private, curative medicine. Some Latin American schools had taken up this subject with vim, and we found classroom, out-patient clinic, and field work in which there was a heartening attempt to integrate the subject of preventive medicine with clinical medicine. It included efforts to point out that "modern medicine" had a far closer relationship to community problems of illness than might be evident at first glance, that physicians should concern themselves more with the circumstances under which people become ill and are likely to become ill again, and with the new concept of "clinical epidemiology." This is a complete turn-over from the previous tradition that "public health and preventive medicine" had to do almost solely with environmental sanitation, water supply, administrative problems of a nonclinical nature, public medical care, health insurance, and large-scale vaccination campaigns.

To show the extent to which efforts were being made along these lines in two medical schools, namely, the one in Cali, Colombia, and the one in El Salvador, some of the courses to be taught by their Departments of Preventive Medicine are listed[7]:

[7] Renjifo-Salcedo, S.: The teaching of preventive medicine and public health in the School of Medicine, University of Valle, Cali, Colombia. *J. med. Ed.*, 1958, *33*, 625.

1st	Year	Medical statistics and general and human ecology.
2d	"	Microbiology, and its application. As an adjunct to formal teaching, a series of home visits was carried out by each student with assignments for microbiological field work within a given family environment. Social anthropology.
3d	"	Preventive medicine in family groups, i.e., social factors in health and disease, the impact of "ways of living" on disease prevalence as judged by the students on their visits to families where they perform immunizations and read skin tests, etc.
4th	"	Preventive aspects of disease. Epidemiology: urban and rural.
5th	"	Public health problems of the community.
6th	"	Internship, which included assignments at local health centers both in urban and rural locations.

Medical research. Examples of excellent research in the medical sciences can be found in South America and Mexico. Some are the results of individual investigations, others of team work. Few follow any particular pattern. It would seem logical to turn first to the government institutes to discover what kind of medical research is being carried out and what its calibre might be. With a few notable exceptions, however, this proved disappointing. A special experience was to find a large building, perhaps a magnificent building with a large staff of professional and technical workers. But within the building one soon became aware of many empty and dusty rooms, and a story of inadequate funds and that the director, a political appointee, "was not in today" because he had two other jobs, one, a desk assignment at the Ministry of Health and the other, "private practice"! Such a situation is not unique, of course, for Latin America.

In some of the medical schools the situation was quite different, a program of interesting research was under way, laboratories were well equipped and full of eager workers. On the other hand, certain eminent research workers, who held or had held important medical school positions, actually carried on their investigations, often with the aid of a team, quite apart from the medical school, either in a small hospital or even a private building. The introduction of the full-time system into a certain number of the medical schools in Latin America should allow for greater research facilities and opportunities so that those faculty members who are equipped to do research can go ahead with their programs. But this is a somewhat delicate situation, perhaps because "medical research" is so easily exploited today and is in such a stage of transition. In some academic surroundings "research" has

FIG. 1. Faculty of Medicine, University of Brazil, Rio de Janeiro, Brazil.

FIG. 2. Faculty of Medicine, University of Montevideo, Uruguay. The partially obstructed placard on the picket fence deals with student demands.

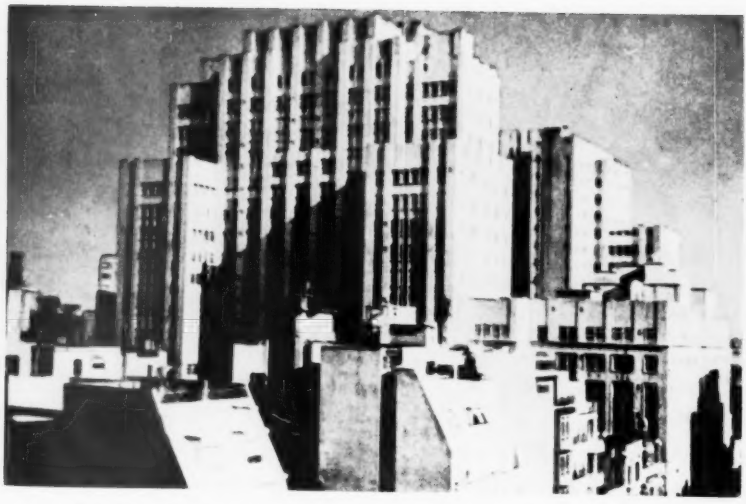

FIG. 3. Faculty of Medicine, University of Buenos Aires, Argentina.

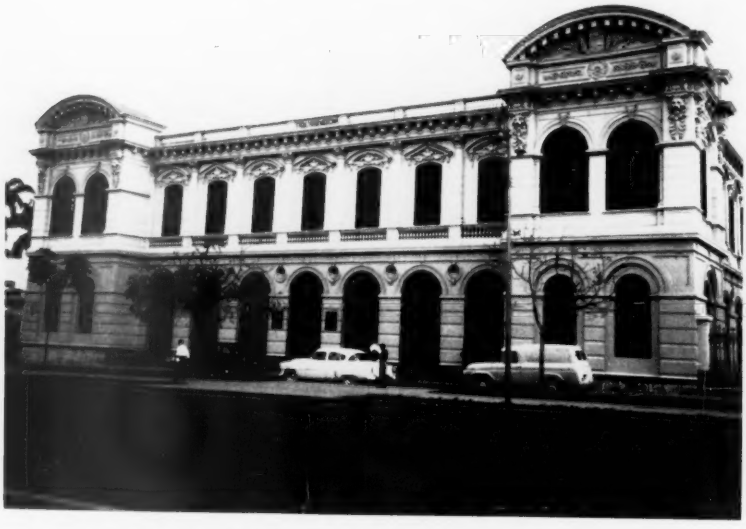

FIG. 4. Faculty of Medicine, San Marcos University, Lima, Peru.

retained the earlier atmosphere of the scholar's study, but in others, "research" has become part of a large technological and cooperative kind of undertaking considerably influenced by current fashions or by the popularity of this or that method. In Latin America, as in North America, the concept of modern medical research has been presented to some of the schools somewhat prematurely as something highly desirable which they must have and something also that seems to be a sort of commodity inseparably associated with a grant for equipment and money for salaries. For this attitude the U.S. granting agencies have been in no small way responsible. At present there is ample evidence of the generosity of these agencies, some of which almost seem to have come in competition with one another. A common defect in Latin America, as elsewhere, is that there has been a casual survey of the situation and money has been both granted and accepted without there being a real capacity on the part of the recipient to meet the responsibilities which medical research may entail. It is understandable that any faculty of medicine, particularly if it finds that more than one agency seems anxious to support its research potentialities and is willing to equip its laboratories, will usually accept these assets. All parties are likely to react initially to a situation of this kind with the feeling of genial satisfaction that a good investment has been made and progress is at hand. And perhaps it is—if we do not draw the line too finely between research and development. But how readily the whole idea can be misunderstood! How easy it is to be led to believe that research and all its outward accoutrements are one and the same, and consist of a building or room which for several years has been shining with new apparatus, or of a protocol for a promising project, which many advisers may have reviewed and approved. As one professor of medicine in Colombia put it: "My country has gone in a few years from the mule to the aeroplane, so please do not expect too much of us all at once, let us catch up with ourselves."

The above comments are not spoken in disparagement. Patience will be required for some of these new facilities in Latin America to bear fruit. Eventually the whole level of opportunities for good and original work may be raised. The actual discoveries may come from totally unforeseen quarters but it is proper to consider that good will result from this great effort to give potential scientists a better chance to show what they can do.

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I. VARIANTS AND THE USE OF SODIUM AZIDE IN THEIR PROPAGATION†

Microbiologists with diverse interests are currently investigating, by varied approaches, the L variants of bacteria. From the cytologist's standpoint, L variants are remarkable in that they lack a cell wall; thus, in their L-stage even rod-shaped bacteria tend to become spherical. Also, the major force which regulates size is removed by this lack of a wall, and particles varying in size from 0.1 μ to 25 μ may exist in the same L colony.[7] The smaller particles may spontaneously pass through a cellophane membrane, thus providing a method for separating the L from the bacterial form when such a mixture exists.[16, 28]

Many studies of L-type variation concern their physiology. The ability to synthesize cell wall is usually reversible, and most variants, when removed from the agent which forced the L-type growth, will again develop walls and thus resume the bacterial state.

Growth requirements of L variants differ markedly from those of the parent strain. Up to the present it was believed that high protein, usually supplied by from 10 per cent to 30 per cent serum, was necessary in the culture media. Now, to the contrary, recent reports[21, 22] emphasize that serum is not required in synthetic medium and that serum serves to detoxify[19, 21] certain substances in natural media rather than to supply essential nutrient. At least in the species studied in this respect it appears that L variants are fastidious, not so much in their demands as in their tendency to be inhibited by certain factors in complex media. The medium of Medill and O'Kane,[21] which contains only vitamin-free casamino acids, salts, lactic acid, and glucose, has supported growth of L forms of four species of *Proteus*[21, 18] and of *Salmonella typhimurium*.[20] L variants of *E. coli* have been cultivated on a similar medium.[18]

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A highly important recent finding shows that for growth, L variants often require hypertonic conditions as provided by a high salt content[26] or 10 per cent sucrose,[13, 14] and that even different strains within a species may vary in their sensitivity to osmotic conditions.[3]

The oxygen requirements of an L form may be the reverse of those needed by the parent culture from which it is derived. The L variants of Salmonella species[8] and of *E. coli*[24] prefer anaerobic conditions and will not grow aerobically in most media.

L variation may play a role in infectious disease, since the L variant of a bacterium may be produced by antibodies[4] or by antibiotics[6] and in most instances is antibiotic resistant. Although L variants tend to be non-pathogenic,[6, 10] the reversing of a pathogen to and from the L stage may be one causative factor in the relapse of infections. *In vivo* production of L variants has been observed by Grasset and Bonifas.[11]

Immunological examinations of L type cultures indicate that the L variant contains many of the same antigens as does the bacterial stage, but not the complete antigen complex.[5, 20] As would be expected, the H antigen is lacking and some of the O antigens also are absent.[27]

The size of a colony of the L form varies with the bacterial species, but it is always much smaller than in the bacterial stage. The L colonies of *Proteus mirabilis* may measure 0.5 mm. in 48 hours and thus be macroscopically visible; for most species studied, the L colony is microscopic.

Presumably because of the lack of a cell wall, the L colony does not stain well with aniline dyes. The dyes employed in staining erythrocytes or protozoa are used for revealing L variants. Dienes' stain,[9] containing both azure II and methylene blue, is commonly used.

A link between protoplasts and L variants has also been sought. Protoplasts are cells stripped of wall substance by a lytic agent, commonly by lysozyme. Protoplasts continue growing for about six hours only, and during this time they may undergo a single multiplication. The dumbbell forms observed represent dividing cells. The majority of cells, not dividing, are nearly identical in diameter, neatly rounded, and several times larger than the bacterial cells from which they originate. Excellent photographs of protoplasts have been published.[15] The cell membrane and possible remnant traces of the cell wall stain intensely with vital stains. Several rod-shaped internal structures are also easily stained. Recent studies indicate that protoplasts may exchange genetic material with each other and with bacterial rods.[14] Protoplasts may still serve as sites for the attachment of certain phages[14] and when cells are infected with phage before protoplast forma-

tion, phage synthesis will continue despite changes involved by protoplast production.[10]

Evidence which indicates that a protoplast is an arrested stage in the transition to the L form is accumulating rapidly. Whereas L variants require agar, protoplasts are usually produced in a liquid medium; and when produced by the action of penicillin in liquid they may progress to form L colonies if transferred to agar-containing medium.[13] Protoplasts may, however, be found in agar medium if conditions are unfavorable for continuing L colony development. On medium buffered at pH 8.0, slightly more alkaline than suitable for L type growth, protoplasts of *Proteus rettgeri* may appear in the presence of penicillin in gradient concentrations.[20] Also with penicillin as the lytic agent, abundant protoplasts have been observed when *Salmonella typhimurium*[20] was cultured on medium containing agar in concentrations excessive for L type growth.

It has thus been shown that protoplasts may progress to L colonies, and that one of the best inducers of L variation, penicillin, is also capable of producing protoplasts from many species: *P. rettgeri, P. mirabilis,*[13] *E. coli,*[13, 14] *Salmonella typhimurium, Salmonella enteriditis.*[28]

The relationship of L variants to the pleuropneumonia-like (P.P.L.O.) forms is not clear. Are the agents of bovine pleuropneumonia and the other P.P.L.O. strains merely L variants which have lost the ability to return to the bacterial stage? Freundt, who has investigated the characteristics of over 200 isolates of P.P.L.O.,[9] has compared such organisms with L variants and concluded that their morphological differences preclude any basic similarity.[8] On the other hand, Kandler and Kandler[12] have reported that with the methods they employed the mode of reproduction (the pinching off of extruding buds) was identical for both types of organisms. An antigenic relationship between certain P.P.L.O. strains and diphtheroids has also been found.[29]

The research here reported concerns a method of stabilizing L growth, permitting its study *in vitro* without its reversion to the bacterial stage. The stabilizing agent, sodium azide, selectively inhibits Gram-negative rods, and this study is concerned only with the Enterobacteriaceae. However, the principle involved, combining a labile substance which induces L variation with a second stable agent which prevents growth of the bacterial stage, should prove useful in maintaining L growth from many types of bacteria.

When L variants are produced by antibiotic action they tend rapidly to revert to the bacterial form as the antibiotic degrades. Even when pure cultures of L variants are obtained by filtration through cellophane mem-

branes, many species soon revert to the rod form, often before complete development of L colonies.

The culture of L forms of certain Enterobacteriaceae in the presence of azide was studied to determine whether sodium azide could prevent reversion of L colonies to the bacillary type. It was believed that a synergistic action might be exerted by azide and penicillin and that therefore less penicillin would be required to produce variation.

MATERIALS AND METHODS

The bacteria studied included three strains of *Proteus mirabilis*, and one each of *Proteus vulgaris, Proteus rettgeri, Proteus morgani, Salmonella typhimurium*, and *Shigella alkalescens*.

Bacteria for inocula were grown in nutrient broth for preliminary tests and on nutrient agar for all later work.

Sodium azide was tested in concentrations from 0.0004 M to 0.004 M.

Tryptose Blood Agar Base (Difco) and P.P.L.O. agar (Difco) were used as basal media. These were enriched with either P.P.L.O. Serum Fraction (Difco), 20 per cent human serum or 20 per cent horse serum. The sera were inactivated at 56° C. before use.

Initial tests employed slide cultures, in which 1.0 ml. of the enriched agar was used to coat a glass slide. After the cultures had been evenly streaked over the surface, a strip of filter paper saturated with bicillin* was placed at one end. This end of the culture was then elevated 2 mm. to facilitate diffusion of the antibiotic. The work specifically described in this paper employed 22 mm. by 50 mm. cover slips instead of slides, which increased resolution of the stained colonies.

After the *modus operandi* for L growth on sodium azide medium had been established, it was found expedient to make cultures on plates, inoculating four or more sectors successively with $\log_{10}$ dilutions of the culture. The organisms were streaked over the surface of the medium and bicillin in a filter paper disc was placed in the center of the plate to diffuse into each sector.

L colonies and bacillary growth were studied with oil immersion objective after staining with Dienes' method. Slide cultures permitted examination and photography of colonies *in situ*. For photography with high magnification a 1 per cent alcoholic solution of basic fuchsin was a superior stain not absorbed by agar.

RESULTS

With concentrations of sodium azide less than 0.003 M, swarming of *Proteus* rods occurred, and thus Azide Blood Agar Base (Difco) which contains this concentration of azide was chosen for the investigation of factors necessary for L growth on azide medium.

When diluted broth cultures were employed, as are commonly used in L variant work with *Proteus*, no L variants were obtained. Such a negative

* Combined dibenzylethylenediamine dipenicillin G, procaine penicillin G, and potassium penicillin G. Wyeth Laboratories, Inc., Philadelphia, Pennsylvania.

series is shown in Table 1. On the azide medium the bacillary growth did not invade the inhibition area, but no L forms appeared. Studies with such moderate inocula were done with all six strains of *Proteus,* none giving L variants on the azide medium.

Large numbers of organisms, taken from solid medium, did give L variants in the presence of sodium azide, as demonstrated by Table 2.

TABLE 1. SODIUM AZIDE MEDIUM WITH MODERATE INOCULUM*

Penicillin	*Control, no sodium azide*	*0.003 M sodium azide in medium*
30,000 units	Large vacuolated colonies 2½ cm. from antibiotic; smaller L colonies to end of slide.	No L colonies; no bacilli.
3,000 units	Large L colonies start 2 cm. from antibiotic, extend to end of slide.	No L colonies; no bacilli.
300 units	As above, except L colonies appear 1½ cm. from antibiotic.	No L colonies; no bacilli.
30 units	L colonies appear 1 cm. from antibiotic. They lack penetrating central growth seen on slides with higher antibiotic concentration.	No L colonies; bacillary growth over entire slide.
3 units	No L colonies. Bacillary growth starts 2 mm. from antibiotic source.	No L colonies; bacillary growth over entire slide.

Inoculum = 10,000 *Proteus mirabilis* per slide.
Eight days' incubation at 37° C.

* Even an excess of antibiotic produces no L variation on sodium azide medium if the inoculum of organisms is moderate.

Typical L colonies, usually of the type with vesicular periphery, were consistently obtained with the three strains of *P. mirabilis* tested.

As soon as it became established that a specific large number of organisms must constitute the inoculum, a change was made to plated medium, in which several inocula could be tested simultaneously in different sectors of the same plate. Table 3 shows the advantage of examining more than one concentration of organisms. The section with 200,000 organisms gave isolated L colonies which were ideal for subculturing; other sections either had confluent L growth, or in the more highly diluted inoculum at times no L growth was formed. Sectored plates were similarly made with *P. morgani, P. rettgeri, P. vulgaris, S. typhimurium,* and *Sh. alkalescens,* revealing in each instance that it was possible to obtain L colonies which would not be

overgrown by swarming bacilli and which would not revert to the bacterial form. Such L colonies may be stored under refrigeration for at least two months without bacillary invasion or reversion of the L phase to the rod stage. Predetermination of ideal inoculum, which varies with strain as well as species, was not required when such sectors were made.

TABLE 2. SODIUM AZIDE MEDIUM WITH HEAVY INOCULUM*

Penicillin	*Control, no sodium azide*	*0.003 M sodium azide in medium*
30,000 units	Results similar to findings with smaller inoculum (see Table 1) except L growth is 1-4 mm. closer to antibiotic.	Large L colonies start 1½ cm. from antibiotic, and extend to end of slide. No bacilli.
3,000 units	Results similar to findings with smaller inoculum (see Table 1) except L growth is 1-4 mm. closer to antibiotic.	Large L colonies start approximately 1 cm. from antibiotic. No bacilli.
300 units	Results similar to findings with smaller inoculum (see Table 1) except L growth is 1-4 mm. closer to antibiotic.	No L colonies. Bacillary growth begins 1 cm. from antibiotic.
30 units	Results similar to findings with smaller inoculum (see Table 1) except L growth is 1-4 mm. closer to antibiotic.	Bacillary colonies only, over entire slide.
3 units	No L colonies. Growth of bacilli over entire slide.	Bacillary colonies only, over entire slide.

Inoculum = 2,400,000 *Proteus mirabilis* per slide.
Eight days' incubation at 37° C.

* L variants are produced on sodium azide medium when the bacterial inoculum is large.

Earlier studies without azide[19] had shown that all of the *Proteus* strains could produce L colonies when P.P.L.O. serum fraction was the added protein. When azide was in the medium the growth requirements became more strict and with the exception of one strain of *P. mirabilis* all of the cultures required horse serum which was definitely superior to human serum.

Increasing the tonicity of the medium by addition of 10 per cent sucrose did not change the antibiotic requirement or the need for a large bacterial inoculum.

For *P. mirabilis* strains two types of L colonies were seen, one vesicular with a dense center (the so-called "fried egg" colony), the other composed of irregular granules. Their appearance was similar with and without azide. For other bacterial species the L stage consisted of sheets of vacuolated growth, enormous vacuolated colonies without differentiated center, or the granular type of colony.

TABLE 3. SHIGELLA ALKALESCENS, VARIED INOCULA ON ANTIBIOTIC DIFFUSION PLATE*

Inoculum	*Growth after 7 days' incubation*
2,000,000 organisms	Sheets of L growth centrally. Small rim bacillary growth peripherally.
200,000 organisms	Isolated L colonies centrally. Bacillary L growth peripherally.
20,000 organisms	No growth, L or bacillary.
2,000 organisms	No growth, L or bacillary.

Medium, Azide Tryptose Agar.
60,000 units penicillin in central disc.
Seven days' incubation at 37° C.

* Varied inocula on same plate give optimum isolation of L colonies.

DISCUSSION

Culture of the organisms on azide medium without antibiotic revealed that while bizarre thick rods often developed, there was no marked central swelling typical of beginning L variation. Thus it would appear that the toxic effect of azide is not directed even partially toward interruption of cell wall synthesis, as is true with penicillin. Therefore, the lack of synergism of azide and penicillin in relation to L variant formation is not surprising.

The increment in penicillin requirement is probably due to the inactivation of the antibiotic by sodium azide, as found by Marco and Buffi.[17] Azide also inhibits many organisms which otherwise might make the change to L growth, and thus the need for a larger inoculum may be explained.

Additional studies of Salmonella isolates have revealed that many strains require a different basal medium for L development than the ones used in this work.

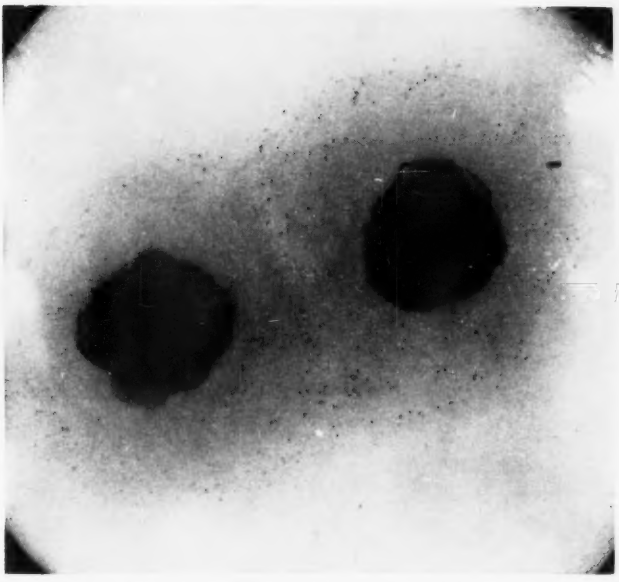

FIG. 1. Typical L colonies of Proteus mirabilis, x110.

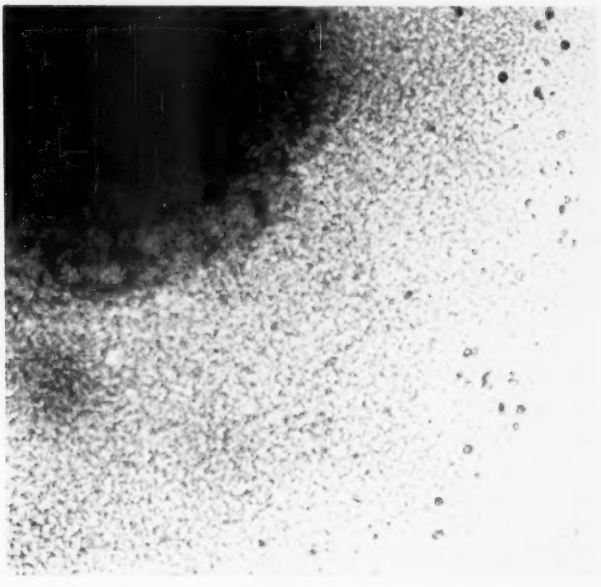

FIG. 2. Edge of one of the colonies shown in No. 1, x1100.

An incidental observation in this laboratory has been that azide medium is useful in isolating P.P.L.O. strains from cervical and urethral specimens, preventing overgrowth by Gram negative rods in the normal flora. Comparative studies will be required before it can be stated that there is no inhibition of P.P.L.O. strains by azide.

SUMMARY

The nature of L variants is reviewed and their relation to protoplasts and the P.P.L.O. forms is discussed.

Experimental work is reported showing that it is possible to prevent reversion of the Enterobacteriaceae L stage to the bacillary form by incorporating sodium azide in the culture medium.

In addition to usefulness in cultivation of L variants, sodium azide medium proved functional for isolation of P.P.L.O. strains from urethral and cervical specimens.

ACKNOWLEDGMENTS

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THE ROLE OF THE ENDOCRINE GLANDS IN KETOSIS—I. THE KETOSIS OF FASTING†

INTRODUCTION

The influence of the pituitary body on fatty acid and ketone body metabolism has excited interest ever since Anselmino and Hoffman demonstrated the ketogenic effect of an anterior pituitary extract in 1931.[1] The extensive literature which followed has been reviewed in two recent publications.[10, 14] The development of ketosis is generally assumed to require an intact adenohypophysis,[30] and both the adrenal and pituitary are said to play vital roles in the mobilization of fat to the liver under a variety of circumstances.[34] Although ketogenesis has been well studied and found to be diminished in the hypophysectomized-depancreatized animal,[47, 57] surprisingly few data are available on the fasting hypophysectomized animal without diabetes and these were obtained from animals fasted only 18 to 36 hours[4, 9, 58] or from fat-fed animals.[48] Some of those reports suggested that the fasted hypophysectomized animal could develop ketonemia, although it was generally agreed that the degree of ketonemia was less than normal or delayed in its onset.

There are even fewer data concerning the relative roles of pituitary hormones and endocrine target gland hormones in the ketosis of fasting in the hypox animal. Bondy and Wilhelmi[9] investigated this subject, but their data on the effect of various hormones upon ketogenesis in liver slices from hypophysectomized rats were obtained from animals fasted for only 18 hours.

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Therefore, it was decided to re-investigate the question of whether the presence of the adenohypophysis is essential to the ketosis of fasting in the rat, employing a more prolonged fast than reported by others. In addition, the experiments were designed to help clarify the inter-relationships among various hormones both in the production of ketosis during the fast and recovery therefrom as the fast is prolonged.

MATERIALS AND METHODS

Male albino rats of the Sprague-Dawley strain weighing approximately 200-300 grams were studied during a 7-day fast in a room maintained at 80° F. Prior to the fast they received Purina Dog Chow, ground or as pellets, and water ad libitum. Rats were free of obvious infection and body weights were stable or increasing at the time of study. Adrenalectomized and hypophysectomized-adrenalectomized animals were maintained with daily injections of 0.5 mg. DOCA subcutaneously. Weight loss following adrenalectomy was regained by the 4th postoperative day and before beginning the fast. Completeness of adrenalectomy and absence of accessory adrenal tissue were later confirmed by failure of the rats to survive without DOCA or NaCl and by post-mortem examination. Hypophysectomzed rats, commercially obtained* were studied either "early" (11-58 days) or "late" (more than 77 days) after hypophysectomy. Completeness of hypophysectomy was judged by failure to grow, testicular atrophy, changes in body hair, decreased spontaneous motor activity, and exquisite sensitivity to insulin (whenever tested). Only rats fulfilling these criteria were used, and adrenocortical atrophy was verified at the time of adrenalectomy or autopsy in every instance. In some experiments hypophysectomized rats received 0.25 mg. cortisone acetate in saline subcutaneously every day. Triiodothyronine, when used, was injected subcutaneously in a dose of 1 μg./100 gms. of body weight daily for 3 days prior to the fast, then 0.8 μg./100 gms. daily during the fast. A solution of L-triiodothyronine was prepared as outlined by Heming and Holtkamp.[59] Distilled, demineralized water was used to avoid inactivation of the hormone by chelation with traces of heavy metals. The solution, freshly prepared every 5 days, was biologically active, judging from the increase in motor activity and body temperature of the hypophysectomized rats which uniformly occurred. Intact rats receiving phloridizin were injected subcutaneously daily with 100 mg. of phloridizin suspended in 1 ml. of sesame oil, a regimen producing profuse glycosuria. Control experiments with phloridizin in sesame oil or sesame oil alone were carried out in nephrectomized rats.

All animals were kept in individual cages through the experiments. Blood specimens were obtained without anesthesia from the tail at approximately the same time each morning and analyzed in duplicate for ketone bodies by the method of Greenberg and Lester as modified in our laboratory[64] and for glucose by the Somogyi-Nelson method.[66]

RESULTS

Tables 1 and 2 compare the patterns of glycemia and ketonemia respectively during a 7-day fast in intact control rats and in rats in which endocrine functions had been modified in various ways.

* The Endocrine Laboratories, Madison, Wisconsin.

TABLE I. GLYCEMIA DURING A SEVEN-DAY FAST

Experimental group	*Day of fast*							
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*
	Blood sugar, mg./100 ml.							
Intact controls	113±4.1 (6)	77±3.1 (6)	76±3.2 (6)	88±1.6 (6)	95±1.6 (6)	98±3.3 (5)	100±2.4 (6)	96±3.0 (6)
Adrx (4 days) DOCA 0.5 mg. q.d.	94±1.7* (9)	65±2.7† (9)	61±2.5* (9)	68±2.1* (9)	56±3.0* (8)	48±3.1* (5)	47±1.7* (4)	
Early hypox (11-58 days)	100±3.5‡ (5)	61±1.2* (5)	57±4.4* (5)	45±4.9* (5)	53±4.0* (5)	42±4.2* (5)	43±4.7* (5)	41±7.6* (5)
Late hypox (135-165 days)	94±4.5† (6)	70±2.9 (6)	65±2.4‡ (6)	53±2.3* (6)	50±2.5* (6)	49±2.7* (6)	44±2.3* (6)	42±5.1* (6)
Late hypox (105-120 days) Cortisone, 0.25 mg. q.d.	106±2.5 (7)	92±2.6* (7)	84±6.0 (7)	92±5.8 (7)	102±3.0 (7)	107±3.5 (7)	100±7.0 (7)	87±4.5 (7)
Late hypox (195-210 days) Adrx (21-28 days), DOCA 0.5 mg. q.d.	95±2.9* (6)	65±3.3‡ (6)	55±2.2* (6)	55±3.0* (6)	47±3.3* (6)	45±3.9* (6)	40±2.7* (6)	38±2.2* (6)
Late hypox Triiodothyronine 0.8 mcg./100 gms./day	100±2.8† (8)	67±2.3† (8)	59±2.0* (8)	49±1.4* (8)	49±3.6* (8)	48±3.6* (8)	37±2.4* (8)	30±2.1* (8)
Intact, phloridizin 100 mg. q.d.	100±4.2 (6)	61±5.1‡ (6)	46±3.7* (5)	50±3.3* (5)				

Data expressed as mean ± standard error.
* $p < .01$ compared to intact controls.
† $p < .02$
‡ $p < .05$

Intact controls vs. other groups

In each instance glycemia and ketonemia in the experimental groups have been compared to the controls and any significant differences indicated. The blood sugar in intact controls fell during the first 24-48 hours of fasting and then increased each subsequent day, until well above the initial fasting level. Blood ketones (Table 2) showed an inverse relationship to the glycemia, with a rise to maximum levels on day 2 followed by a progressive fall below the initial fasting levels. Neither ketonemia nor glycemia was restored completely to levels present in fed rats (day 0). Every experimental group except the "late" hypophysectomized rats maintained with 0.25 mg. cortisone acetate daily demonstrated a strikingly different pattern from that of the controls. In all other groups of adrenalectomized and hypophysectomized rats glycemia progressively declined, and ketonemia increased to higher levels than in the control rats and did not decline as the fast continued. As in the controls, there appeared to be an inverse relationship between glycemia and ketonemia.

"Early" hypophysectomized vs. "late" hypophysectomized rats

In vitro studies have demonstrated that the rate of ketogenesis by liver slices from hypophysectomized rats is depressed, and the degree of impairment varies directly with the time elapsed since hypophysectomy.[1] Our *in vivo* data on ketonemia during prolonged fasting in "early" and "late" hypophysectomized rats did not confirm those *in vitro* results. "Late" hypophysectomized rats were found to develop significantly more ketosis than the "early" group after 4, 6, and 7 days of fasting ($P < 05$, $P < 05$ and $P < 02$) and, as noted above, both groups exhibited much greater ketonemia than the fasted controls. Glycemia did not differ between the "early" and "late" hypophysectomized groups except for day 1 when the former had a slightly lower blood sugar ($P < 05$). The loss of weight by control, early hypophysectomized, and late hypophysectomized rats during the fast is illustrated in Figure 1. Although their initial weight was less, the "early" hypophysectomized rats lost more weight than the "late" group during the fast. One possible explanation for this difference is that the adrenal glands of the former were less completely atrophic, permitting greater nitrogen loss early during the fast. This possibility is supported by the fact that the two curves differ in slope only during the first two days of the fast, when the bulk of the nitrogen loss should occur. In addition, "late" hypophysectomized rats subsequently adrenalectomized, maintained on DOCA, and subjected to a 7-day fast demonstrated a pattern of weight loss almost identical to the "late" hypophysectomized group which had not been adrenalectomized.

TABLE 2. KETONEMIA DURING A SEVEN-DAY FAST

Experimental group	Day of fast 0	1	2	3	4	5	6	7
	Blood ketones, mg./100 ml. (as acetone)							
Intact controls	1.0±0.07 (9)	5.5±0.57 (9)	7.7±0.54 (9)	6.8±0.71 (9)	5.0±0.50 (9)	3.8±0.59 (9)	2.9±0.24 (9)	2.5±0.24 (9)
Adrx (4 days) DOCA 0.5 mg. q.d.	1.4±0.17‡ (9)	6.1±0.57 (9)	9.3±0.42‡ (9)	12.1±0.48* (9)	12.1±0.81* (8)	11.4±1.28* (5)	9.0±1.71* (4)	
Early hypox (11-58 days)	1.7±0.32† (5)	5.3±0.87 (5)	6.2±0.51 (5)	7.8±1.10 (5)	8.8±1.52† (5)	12.2±4.12† (5)	10.1±2.87* (5)	11.2±2.29* (5)
Late hypox (135-165 days)	1.2±0.22 (6)	3.7±0.42‡ (6)	7.7±0.56 (6)	11.9±1.59* (6)	15.6±2.13* (6)	16.9±1.81* (6)	18.9±1.97* (6)	19.4±1.62* (6)
Late hypox (105-120 days) Cortisone, 0.25 mg. q.d.	1.4±0.10* (7)	2.3±0.26* (7)	4.1±0.96* (7)	4.6±1.09 (7)	3.1±0.57‡ (7)	3.4±0.61 (7)	4.7±1.06 (7)	6.5±1.23* (7)
Late hypox (195-210 days) Adrx (21-28 days), DOCA 0.5 mg. q.d.	1.6±0.21* (6)	3.6±0.27‡ (6)	6.9±0.50 (6)	9.4±0.72‡ (6)	12.9±1.28* (6)	19.6±2.54* (6)	22.8±2.43* (6)	26.2±3.46* (6)
Late hypox Triiodothyronine 0.8 mcg./100 gms./day	1.3±0.05* (8)	4.4±0.35 (8)	8.3±0.54 (8)	9.9±0.64* (8)	12.8±1.19* (8)	14.7±1.27* (8)	17.3±1.17* (8)	17.4±1.15* (8)
Intact, phloridizin 100 mg. q.d.	1.1±0.33 (6)	23.3±4.90* (6)	51.1±4.69* (5)	68.5±5.20* (5)				

Data expressed as mean ± standard error.
* $p < .01$ compared to intact controls.
† $p < .02$
‡ $p < .05$

Late hypophysectomized vs. late hypophysectomized-adrenalectomized vs. late hypophysectomized-cortisone treatment

In view of the above differences in weight loss it became important to assess the degree of adrenal cortical atrophy in the late hypophysectomized rats and to evaluate the role of secondary adrenocortical atrophy in their development of marked ketonemia. The situation was confusing since Tepperman and Tepperman[61] reported that both cortisone and growth hormone

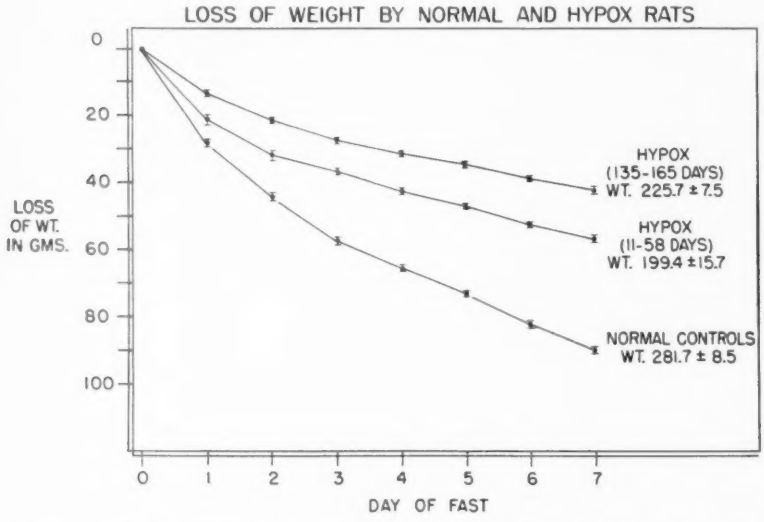

FIG. 1. The mean weight loss ± standard error on each day is indicated for each experimental group. The weight listed under each group represents the body weight on day 0.

were necessary to *restore* ketogenesis in liver slices from "late" hypophysectomized rats, making our results even more unexpected. In order to clarify this issue, some "late" hypophysectomized rats were adrenalectomized while others were given cortisone before subjecting them to the prolonged fast. All of the adrenal glands removed were markedly atrophic.

Statistical analysis of the data of Table 2 reveals that adrenalectomy has no significant effect upon the ketonemia of late hypophysectomized rats. However, treatment of "late" hypophysectomized rats with a small constant dose of cortisone acetate (0.25 mg.) daily restored the pattern of glycemia to virtually normal. Despite the constancy of the cortisone dose, the blood sugar fell to its lowest level on day 2 and then rose progressively, just as in

the controls. Ketonemia also simulated the normal pattern, although not as closely as did glycemia.

The role of the adrenal cortex in the glycemia and ketonemia of the "late" hypophysectomized rats can be better appreciated by inspecting Figure 2. The inverse relationship between glycemia and ketonemia in all groups is readily apparent, as is the failure of adrenalectomy to modify the patterns found in the "late" hypophysectomized rat. The ability of a "permissive"

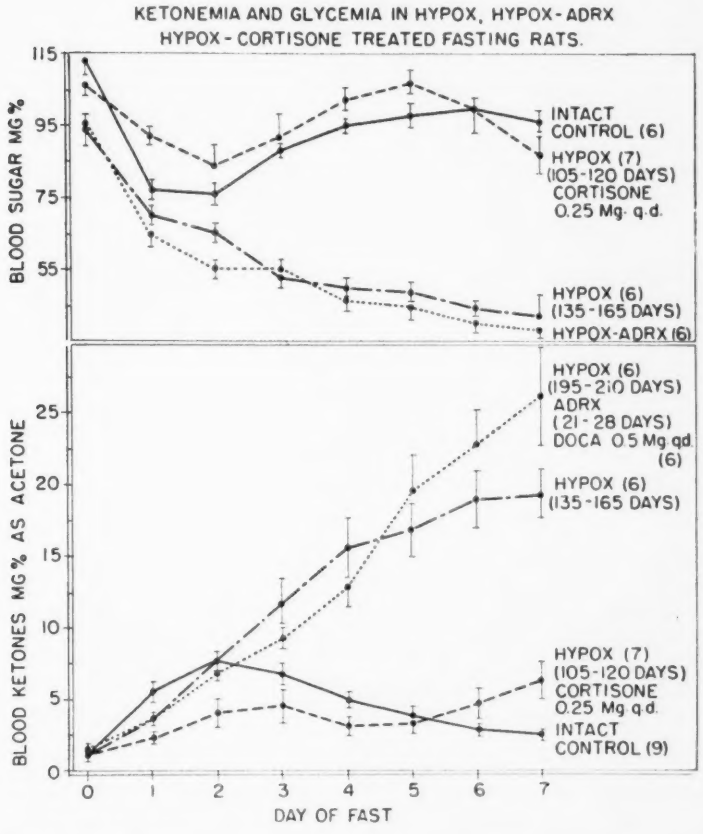

FIG. 2. Each point represents the mean value ± standard error. The number of rats in each experimental group is indicated in ( ). The time elapsed since hypophysectomy or adrenalectomy is recorded in days. Note the inverse relationship between glycemia and ketonemia in each instance.

dose of cortisone to modify that pattern, simulating the response of the intact controls, is evident.

Late hypophysectomized vs. DOCA maintained-adrenalectomized rats

The comparison between these two groups is quite difficult since the adrenalectomized rats did not tolerate the fast as well as hypophysectomized rats despite maintenance therapy with DOCA. They were either dead or showed evidence of peripheral circulatory failure by the 7th day. In all likelihood their condition was probably not as good as that of the hypophysectomized rats even earlier during the fast. Since impairment of the circulation will suppress ketogenesis,[17] this factor alone might account for the lower ketone levels on days 5 and 6 ($P < 05$, $P < 01$). The adrenalectomized rats were more ketonemic than the hypophysectomized on days 1 and 2 ($P < 01$, $P < 05$). Glycemia differed only on day 3 when the adrenalectomized rats demonstrated an unexplained small but significant rise in blood sugar ($P < 01$). The ketonemia in the fasted adrenalectomized rats confirms earlier observations.[18]

Late hypophysectomized vs. late hypophysectomized-triiodothyronine treatment

Bondy and Wilhelmi have reported that thyroxine treatment of hypohysectomized rats will restore to normal the low rate of ketogenesis by their liver slices.[8] For this reason, triiodothyronine was administered to a group of "late" hypophysectomized rats. There was no significant difference in ketonemia compared to that of "late" hypophysectomized rats although the animals treated with triiodothyronine had a lower blood sugar on days 3 and 7 ($P < 02$, $P < 05$).

Intact phloridizinized rats vs. other groups

The apparently greater tendency of the hypophysectomized rat to develop ketonemia during fasting was surprising since many previous well-documented studies indicated that hypophysectomized or hypophysectomized-diabetic animals exhibit less ketosis under a variety of circumstances than do intact or diabetic animals.[30, 47, 52, 53, 57, 61, 67] It is apparent from our data that there was always an inverse relationship between glycemia and ketonemia. The hypophysectomized and adrenalectomized rats always exhibited more profound hypoglycemia than the intact animals, except when the former were treated with cortisone. If the hypoglycemia with its probable concomitant depletion of liver glycogen is the important ketogenic stimulus, it is not justified to assume a priori that the hypophysectomized or adrenalectomized rats have an intrinsically greater capacity to develop ketosis than

do intact rats or conversely that the pituitary gland is not involved in fasting ketosis. To establish the former point would require data from intact rats exhibiting a degree of chronic hypoglycemia comparable to that of the hypophysectomized and adrenalectomized rats. For this reason an experiment was performed in which intact rats were given phloridizin with the production of a comparably hypoglycemic state (Table 1). The spectacular degree of ketonemia obtained is recorded in Table 2 and can perhaps be better appreciated by inspecting Figure 3 which contrasts it with ketonemia in

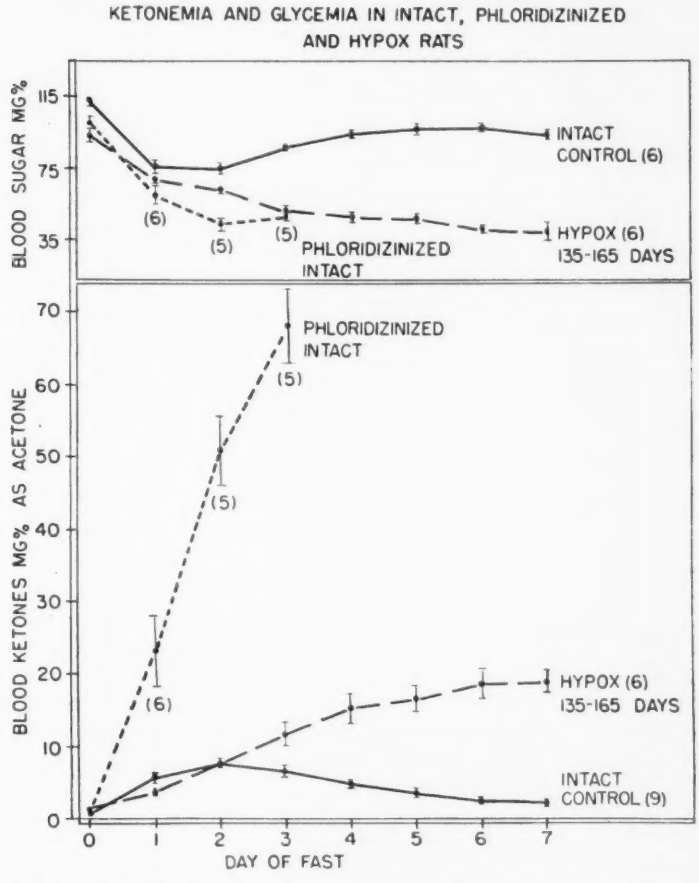

FIG. 3. The number of rats in each group is indicated in ( ). Note the extreme degree of ketonemia in intact phloridizinized rats contrasted to other groups.

hypophysectomized and in intact control rats. In addition, the phloridizinized rats had polyuria and heavy ketonuria (qualitative test) contrasted to the oliguria and minimal ketonuria found in hypophysectomized or adrenalectomized rats. It is apparent that ketosis was many-fold greater in the phloridizinized intact rats than in hypophysectomized rats with a compar-

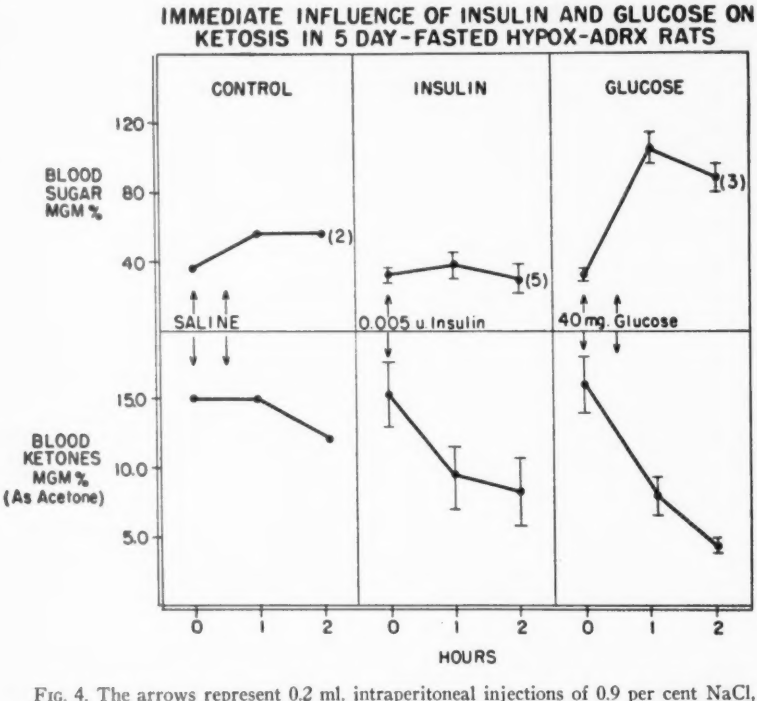

FIG. 4. The arrows represent 0.2 ml. intraperitoneal injections of 0.9 per cent NaCl, glucagon-free insulin in 0.9 per cent NaCl or 20 per cent dextrose in water. Although not indicated by an arrow, the rats receiving insulin also received 0.2 ml. of saline at ½ hour.

able degree of hypoglycemia. The former tolerated the experiment poorly and after the third day rapidly developed circulatory collapse and died.

To investigate the possibility that phloridizin or its sesame oil vehicle might be directly responsible for the ketosis rather than the hypoglycemia it induced, similar studies were performed in nephrectomized rats and phloridizinized-nephrectomized rats. No difference in ketonemia was apparent between those two groups, eliminating that possibility.

Effect of insulin, glucose, or saline in fasted hypophysectomized-adrenalectomized rats

Since both fasting[8] and hypophysectomy[58] are said to cause a decrease in endogenous insulin secretion the possibility that insulin insufficiency plays a role in the ketosis of fasting was investigated. Figure 4 illustrates the data obtained when insulin, glucose, or saline were administered to "late" hypophysectomized-adrenalectomized rats which had been fasting for 5 days. An extremely minute dose of glucagon-free insulin caused a prompt, highly significant decline in ketonemia. The actual dose of insulin administered was probably even smaller than 0.005 unit since the insulin solution was so dilute and had been diluted with normal saline rather than with a solution containing albumin.[6, 20] A somewhat larger, more sustained, decline in ketonemia occurred when glucose was administered, while saline injected as a control was virtually without effect. The latter two groups contained too few animals for adequate statistical evaluation. For the same reason the apparent rise in blood sugar in the rats injected with saline is difficult to interpret.

These data suggest that decreased utilization of carbohydrate contributes to the development of fasting ketosis in the hypophysectomized-adrenalectomized rat, and that the decrease is probably due to deficiency of both insulin and substrate (carbohydrate). Steele, *et al*,[58] have already presented evidence for decreased peripheral carbohydrate utilization in the hypophysectomized dog.

DISCUSSION

The production of ketone bodies and other events in the intermediary metabolism of fatty acids have been clarified during the past several years and are reviewed in detail in a number of recent publications.[10, 14, 55] These emphasize the central position of acetyl CoA in the inter-relationships of fat, protein, and carbohydrate metabolism; the importance of the balance between acetyl CoA and Krebs cycle intermediates in the liver in determining whether ketosis will occur; and the importance of TPNH generated either from the oxidation of glucose via the hexose monophosphate shunt pathway or possibly from the Krebs cycle in determining whether fat synthesis can take place. Even more recently insight has been gained concerning the quantitative significance of nonesterified fatty acids in satisfying energy requirements during fasting and the rapidity with which they can be released from adipose tissue and transported to the liver and other organs to serve as substrate for fatty acid oxidative systems.[13, 21] The effect of hormones upon ketosis or other aspects of fat metabolism must be explained within the

known frameworks of intermediary metabolism and adipokinesis since a basic principle of endocrinology is that hormones do not initiate or stop metabolic reactions but can modify their rates. Since carbohydrate, protein, and fat metabolism are so closely intertwined, a primary alteration in the metabolism of one of those substances may be eclipsed by profound secondary effects on the metabolism of the others. This may create an erroneous impression of the site of action of the hormone under investigation. The study of ketosis is particularly vulnerable to that pitfall since the degree of ketosis may depend more upon the balance between fat and carbohydrate or protein metabolism than upon fat catabolism per se. Despite these and other limitations cited below, some interpretations of the role of the endocrine glands in ketosis are possible.

In the normal rat ketonemia and glycemia varied in inverse relation to each other and exhibited a biphasic response as the fast was prolonged. The peak of ketonemia and trough of glycemia occurred by the second day with gradual recovery thereafter. In contrast, removal of either the adrenal glands or hypophysis led to progressively increasing ketonemia and sustained hypoglycemia. Although the biphasic response to fasting was abolished, the inverse relationship between ketonemia and glycemia persisted. The biphasic response was restored by treatment of the hypophysectomized rat with a "permissive" dose of cortisone.

The data obtained in the adrenalectomized rats confirm earlier observations that adrenalectomized rats maintained in an adequate circulatory status develop even greater ketonemia during fasting than intact controls.[15] Adrenalectomized rats also develop ketonemia after insulin hypoglycemia,[1] which simulates the pattern of prolonged fasting telescoped into a period of a few hours. These results, coupled with the demonstration that treatment with cortisone suppresses fasting ketosis in man[33] and the intact rat,[15, 50] might suggest that the adrenal gland has a direct inhibitory effect on ketosis. However, a review of the literature indicates that the influence of the adrenal gland on ketosis has been a controversial subject.

Some investigators have demonstrated ketosis and/or adipokinesis in adrenalectomized animals during fasting,[62] or after administration of anterior pituitary extracts,[27, 36] purified growth hormone,[7, 39] and oxycel corticotrophin.[8, 16] Others have presented incontrovertible evidence that adrenalectomy impairs the mobilization of peripheral fat to the liver under a variety of circumstances.[22, 34, 49] Although epinephrine has adipokinetic properties,[11, 68] any impaired adipokinesis after adrenalectomy presumably is due to the absence of the adrenal cortex alone since adrenal demedullation does not impair the mobilization of fat.[32, 68] It seems possible that any impairment of ketogenesis in the adrenalectomized animal may be primarily due to im-

paired adipokinesis. However, Tracht, Goldstein, and Ramey[62] have maintained that normal adipokinesis is possible in the adrenalectomized rat. This conclusion is based upon the percentage change in weight of the epididymal fat pad in response to fasting or administration of phloridizin. Their data are difficult to interpret since the absolute initial weight of the epididymal pads and subsequent absolute weight loss were considerably smaller in adrenalectomized than in control rats despite comparable body weights. The liver of the adrenalectomized rat can take up exogenously administered fatty acids and convert them to ketone bodies at a normal rate as has been demonstrated by perfusing the isolated liver with neutral fat emulsions,[28] administering C^{14}-labeled octanoate,[43] or measuring ketogenesis in surviving liver slices.[9]

While the administration of cortisone suppresses fasting ketosis in man[38] and the intact rats,[18, 50] it does not suppress the ketosis which follows insulin hypoglycemia in either species.[1, 38] In the cortisone-treated rat the liver is loaded with glycogen during fasting, while after insulin hypoglycemia it is depleted of glycogen.[1] Moreover, in the hypophysectomized-depancreatized rat[51] and baboon[24]cortisone seems to promote the development of a fatty liver and, in the case of the rat, intense ketosis.[51] Preliminary data in the baboon reveal that ketosis does not occur despite the fatty liver, suggesting that in that species ketosis may be dissociated from its usual metabolic concomitants.[25] The augmentation of ketosis in the depancreatized rat and its suppression in the intact fasted rat suggest that insulin must be present in those situations in which cortisone has an antiketogenic effect.

Since the studies cited have been performed in many different species under a variety of conditions, it is not surprising that different data and interpretations have resulted. Other factors which help to explain the discrepancies are the presence of circulatory insufficiency, which is known to inhibit ketosis,[17] and reliance on the measurement of ketonuria alone, since adrenalectomy diminishes the renal clearance of ketone bodies.[41]

It is obvious that the adrenal gland is not essential to the development of ketosis, but whether adrenal cortical hormones do have a physiologically important direct effect on some phase of fat metabolism cannot be unequivocally answered by these studies. The evidence for possible direct effects has been reviewed in the past.[10] In the current studies, and those in which insulin hypoglycemia was induced,[1] there is an obvious inverse relationship between ketonemia and glycemia or liver glycogen levels. It is tempting to explain the effects of adrenalectomy or cortisone administration on ketonemia as being indirect manifestations of the well-established influence of the adrenal cortex on gluconeogenesis. The presence of an endogenous carbohydrate pool, via gluconeogenesis, would be antiketogenic

by restoring the balance between acetyl CoA derived from fat and Krebs Cycle intermediates, regenerated once again via the carboxylation of pyruvate or oxidative deamination of various amino acids. In the absence of gluconeogenesis, hypoglycemia and ketonemia would continue. However, that explanation cannot explain all of the data. The enormously greater degree of ketonemia found in intact rats made comparably hypoglycemic by administration of phloridizin suggests that the adrenalectomized rat does have an impaired ability to develop ketosis although some degree of ketosis can occur.

In contrast to the conflicting reports on the role of the adrenal in ketosis, it has been generally accepted that adipokinesis and ketosis will not occur in the absence of the adenohypophysis.[20, 22, 24, 27] That view has been strengthened by the fact that injection of anterior pituitary extracts and some "purified" anterior pituitary hormones will induce ketosis in experimental animals.[2, 5, 7, 10, 44, 59, 63] The presence of a separate pituitary ketogenic hormone has not been established. Instead, the available data suggest that individual trophic hormones possess ketogenic activity.[15] The development of greater ketonemia in hypophysectomized rats than in normal rats during a prolonged fast came as a surprise in view of the presumed indispensability of the hypophysis in ketosis. The hypophysectomized rat can also develop ketosis acutely in response to insulin hypoglycemia.[1] Furthermore, although Bondy and Wilhelmi[9] and Tepperman and Tepperman[61] found that the impairment of ketogenesis in surviving liver slices became progressively more severe as time elapsed after hypophysectomy, our "late" hypophysectomized group had even greater ketonemia than the "early" group. Bondy and Wilhelmi[9] found that treatment of hypophysectomized rats with thyroxine restored ketogenesis in the liver slices to normal whereas in the present work no further accentuation of ketosis occurred in the fasted hypophysectomized rats injected with triiodothyronine. However, the present studies in the whole animal are influenced by both ketogenesis and ketolysis and may not be comparable to their work if ketolysis by extrahepatic tissues is increased by thyroid hormone. Geyer, Shaw, and Greep[26] have reported that the oxidation of fatty acids to CO_2 does increase parallel to the increase in general metabolic rate when thyroid hormone is administered.

Our results after administration of cortisone to the "late" hypophysectomized rats also differed from the experience of others who found that cortisone potentiated the effect of growth hormone in repairing ketogenesis in liver slices[61] or restored ketosis in hypophysectomized-depancreatectomized rats.[51] Instead of enhancing ketonemia, the small constant daily dose of cortisone virtually restored the patterns of glycemia and ketonemia to normal. This suggests that the gluconeogenic response to fasting in the in-

tact animal may be another instance of the "permissive" action of adrenal cortical hormones[81] rather than being due to hypersecretion of those hormones.

The general impression that the hypophysectomized animal cannot develop ketosis on fasting is not warranted since the literature does not contain sufficient data to sustain that opinion. Many data are available on ketosis in the hypophysectomized diabetic animal, but similar studies on nondiabetic hypophysectomized animals are few in number and the fasts of brief duration. The widespread belief that hypophysectomized animals cannot tolerate a prolonged fast has discouraged such studies. However, Reinecke, Samuels, and Roberts[46] found that hypophysectomized rats could survive fasts of 3-4 weeks. Since the fast was started only 21 days after hypophysectomy and glycemia was well maintained, their rats probably had incomplete atrophy of their target organs. It is not known whether ketosis developed since ketone bodies were not measured. We were able to find only three reports of blood ketone levels in fasting hypophysectomized rats. Bondy and Wilhelmi found "normal" ketonemia at 18 hours,[9] Shipley[68] definite but subnormal ketonemia at 24 hours and, the longest fast, Bahner and Taylor,[4] normal ketonemia at 36 hours in rats hypophysectomized only 8-12 days. Those results are comparable to our findings at 24 and 48 hours since our hypophysectomized rats were not significantly more ketonemic than the controls until 72 hours had elapsed. Although the present study demonstrates that the hypophysectomized rat obviously can develop marked ketosis during fasting, the data cannot exclude an influence of the hypophysis upon ketosis. The phloridizin experiment clearly shows that when a comparable degree of hypoglycemia is induced in the normal rat the animal's capacity to develop ketosis is tremendously greater than that of the hypophysectomized rat. It seems fair to conclude then, in agreement with earlier workers, that hypophysectomy does indeed depress the capacity to develop ketosis. This defect is even more striking when one compares the pancreatectomized to the pancreatectomized-hypophysectomized animal. In the former, ketosis occurs so rapidly and to such a striking degree that the latter's ketosis, which is of the same order as in the fasting animal, appears inconsequential by comparison.[47, 59] The hypophysis may exert its influence on ketosis by restoring adipokinesis to normal since hypophysectomized rats can utilize exogenously administered fatty acids or neutral fat[23, 25] and since growth hormone and ACTH have been shown to accelerate the release of nonesterified fatty acids from depot fat *in vivo* and *in vitro*.[45, 65] However, a direct effect on ketogenesis in the liver is also possible since various pituitary ketogenic factors have been reported to increase the rate of conversion of infused octanoate to ketone bodies,[10] and have a direct *in vitro*

ketogenic effect on surviving liver slices when added to the incubation mixture.[54, 81] Numerous other attempts to demonstrate an *in vitro* effect on the liver have failed.

It may be inferred from the data presented that in the intact animal pituitary factors do play a role in augmenting ketosis during fasting while the adrenal cortex by "permitting" gluconeogenesis to occur moderates ketosis. An additional factor almost certainly contributing to the development of ketosis is the decline in endogenous insulin secretion during fasting.[8] The hypoinsulinism may be magnified by a decreased tissue sensitivity to insulin,[18] probably due in part to secretion of growth hormone or other pituitary factors. A similar situation must exist in the hypophysectomized animal since hypophysectomy per se will result in suppression of insulin secretion.[24, 58] Our fasting hypophysectomized-adrenalectomized rats exhibited a significant decline in ketonemia in response to an extremely minute dose of insulin, so minute that it did not lower the blood sugar. This suggests that an almost imperceptible degree of utilization of glucose may be enough to exert the sparing effect of carbohydrate upon the oxidation of fatty acids.[35, 87] A similar effect is discernible when 0.05 unit of insulin is administered to intact rats experiencing ketosis initially precipitated by insulin-induced hypoglycemia.[1] In those rats the administration of glucose did not promptly diminish the ketosis as had happened in the fasting hypophysectomized-adrenalectomized rat. It is well known that some utilization of carbohydrate can occur in the hypophysectomized-adrenalectomized animal even in the total absence of insulin, and that utilization undoubtedly was responsible for the decline in ketonemia.

The data obtained from the present study as well as those reported by others permit a tentative formulation of the role of the adenohypophysis and other endocrine glands in the ketosis of fasting. As liver glycogen becomes depleted during the first 24-48 hours of fasting,[39] the resulting declining blood sugar probably triggers the release of growth hormone,[48] ACTH,[60] and perhaps other pituitary factors, all of which may influence the mobilization of fat.[14, 15, 84] These hormones can exert a direct effect on adipose tissue, stimulating the release of nonesterified fatty acids[45, 65] and possibly triglyceride fat as well,[21, 58] leading to an accumulation of lipid in the liver. Epinephrine and nonepinephrine may also be involved in this reaction.[11, 66, 68] Insulin secretion declines during fasting[8] and that decline and the decreased availability of carbohydrate both play a major role in the suppression of lipogenesis in the liver and adipose tissue. These events set the stage for the utilization of fatty acids to satisfy energy requirements in the glycogen depleted, Krebs Cycle deficient liver and ketosis results; a sequence similar to that postulated by Mirsky in 1936.[40] By the end of the third day, the ACTH

activated adrenal cortex has "permitted" gluconeogenesis to occur at a rapid rate from glycogenic amino acids obtained by accelerating the catabolism of endogenous protein. Ketogenesis is suppressed by the restoration of the balance between acetyl CoA and the Krebs Cycle by the regeneration of Krebs Cycle intermediates.[5] These may be regenerated either via the carboxylation of newly formed pyruvate or more directly by the oxidative deamination or transamination of the appropriate amino acids. In addition, the oxidation of fatty acids may be spared to some extent by the availability of endogenously formed carbohydrate,[35, 37] although fat is still the major metabolic fuel. As those processes continue, glycemia progressively increases and ketonemia progressively declines. None of the hormones are essential to the development of ketosis but their presence or absence markedly affects both the rate of development and degree of ketosis. The thyroid probably has a nonspecific effect on the rates of virtually all reactions while insulin and the pituitary and adrenal hormones must have more specific effects on rates of various reactions although the specific sites and mechanisms of action are not known. The ACTH-activated adrenal cortex appears to play an important role in the reduction of fasting ketosis via gluconeogenesis and seems to exert its effect in a "permissive" fashion, allowing some phase of protein catabolism to occur at a physiologically important rate.

It is apparent that while some degree of fasting ketosis can occur relatively independently of the endocrine glands, the magnitude of the ketonemic response and the recovery from ketosis are both importantly influenced by insulin, the adrenal cortex, and the adenohypophysis.

SUMMARY AND CONCLUSIONS

The influence of various endocrine factors on ketosis was studied in male rats during a 7-day fast. Maximal ketonemia in control rats occurred within 2 days, then declined progressively. Glycemia fell to its lowest level while ketonemia was at its peak and then rose well above the initial fasting baseline. The observation that the ketonemia of fasting is greater in adrenalectomized than in control rats was confirmed. Fasting hypophysectomized rats also developed greater ketonemia than the controls. Ketonemia was more marked in rats studied several months after hypophysectomy than in rats only a few weeks' post-hypophysectomy. Neither adrenalectomy nor triiodothyronine significantly altered ketonemia in "late" hypophysectomized rats, but daily administration of a "permissive" dose of cortisone altered both glycemia and ketonemia, simulating the normal pattern. When sustained hypoglycemia comparable to that of hypophysectomized or adrenalectomized rats was produced in fasting intact rats by the administration of phloridizin, the latter developed a degree of ketonemia far greater than

that in the former. Phloridizin was not ketogenic in the nephrectomized rat. A rapid, striking decline in ketonemia occurred in the fasted hypophysectomized-adrenalectomized rat when either insulin or glucose was administered.

Neither the adrenals nor the hypophysis are essential for the development of fasting ketosis in the rat but both influence the magnitude of the response. With comparable degrees of hypoglycemia the fasted intact rat develops much greater ketosis than the hypophysectomized and/or adrenalectomized rat, indicating a role for these glands in promoting ketosis. On the other hand, the decline in ketonemia during continuation of a fast in intact rats is probably mediated through the adrenal cortex and may be a "permissive" effect of adrenal steroids. The contributory role of a decline in insulin secretion during fasting is also to be considered. The biochemical mechanisms involved in the ketogenic-antiketogenic actions of various hormones are discussed.

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ADDENDUM

The article by Dr. Hans Lenz entitled "Electron microscopic studies on the organic matrix of enamel and dentin" which appeared in the December 1958 issue of the Journal should have carried a note thanking the National Institutes of Health, U.S. Public Health Service, for a grant which entirely financed the printing of the electron micrographs illustrating the paper.

EVENTS

RADIOTHERAPY SEMINAR

March 19, 1959

RADIOTHERAPY OF THYROID CANCER. By Dr. Virginia Kneeland Frantz, Department of Surgical Pathology, Columbia University College of Physicians and Surgeons, New York City.

Grade III carcinomas of the thyroid are extremely anaplastic and most are rapidly fatal. A slight response to radiotherapy is rarely seen. Death usually results from asphyxia (choking), hemorrhage, or concurrent infection. Grade II carcinomas are not as anaplastic but show an unpredictable clinical course.

Grade I carcinomas comprise 75 per cent of all thyroid cancers. There are two main types: the papillary type, which is nonfunctional, and the malignant adenoma, which is functional. A "radium collar" was used to treat these postoperatively in the past. Now it is felt that disease in the neck with metastases to cervical (and mediastinal) nodes should be treated by radical surgery, total thyroidectomy, cervical lymph node dissection, and dissection of the mediastinum if necessary. Radiotherapy should be reserved for distant metastases.

The papillary type is most common; survival time is good, even with previous inadequate treatment, but late recurrences and more malignant behaviour do occur. Early metastases to cervical nodes are the rule, especially in young people; this type is usually bilateral and liable to be multifocal.

The malignant adenoma is only occasionally locally aggressive but disseminates by early cryptic metastases to bone, often later than presenting symptoms. This type shows no local recurrence after its removal and appears benign. Radioactive iodine uptake in metastases of this type of tumor can be enhanced by ablation of the entire thyroid gland by surgery or radiotherapy. Thyroid hormone can also enhance the uptake of radioactive iodine as can thiouracil, but use of the latter leads to intolerable hypothyroidism. Calcification can occur in both types of tumor in the primary site.

A thyroidectomy is indicated in both types *at the time* of operation if a diagnosis can be reached by the use of frozen sections. Biopsy followed by thyroidectomy later, through scar tissue, increases the risk of recurrent laryngeal paralysis and hypoparathyroidism. Radical neck dissection should be performed on the side of the affected lobe. Paraglandular and paratracheal nodes sometimes can be used as indicators for a radical dissection of the opposite neck. In some hospitals a bilateral radical neck dissection is routine, but this can lead to permanent facial edema.

In radiotherapy of thyroid cancer, X-ray uptake can sometimes be indicated by radioautographs of the primary tumor. Most radioactivity is found in the colloid.

Nontoxic nodular goiter can be confused with benign follicular adenoma. The latter neoplasm also occurs in cretins and acromegalics, where it may be mistaken for adenoma malignum. In a follicular adenoma, small uniform

follicles are seen throughout the tumor, but the uptake of radioactive iodine, indicating sites of hormone production, may be patchy. Radiotherapy of this type of tumor is not wholly effective, since the effective radiations here are not the measured γ-rays, but B-rays, which have an effective radiation distance of only two millimeters.

Carcinoma is seen in 6.6 per cent of cases of nontoxic nodular goiter and comprises 21 per cent of all thyroid neoplasms. It is even more common in young males. Carcinoma is quite rare in cases of toxic goiter.

In a ten-year follow-up of 177 thyroid cancers treated with radiotherapy, the incidence of histological types was as follows: papillary type, 45 per cent; malignant adenoma, 32 per cent; grade II, 11 per cent; grade III, 10 per cent. The papillary type is relatively insensitive to X-ray therapy, and, due to lack of function, there is a corresponding lack of radioactive iodine uptake.

Some tumors metastasize in both functional and nonfunctional forms; this type is known as a "mixed" type but is usually predominantly papillary. It is locally aggressive and metastasizes to lymph nodes and lungs, but blood vessel invasion and growth within the lumen of large vessels can occur without distant metastases.

D. W. B.

ZOOLOGICAL JOURNAL CLUB

April 6, 1959

PROTEIN PERMEABILITY STUDIES IN THE CHICK EMBRYO. By Patricia F. Knight, University of California, Berkeley.

Before studies of the intracellular effects of exogenous macromolecules can be attempted, it is necessary to determine whether or not such molecules can pass into cells without being altered or degraded. Experiments with this end in view, using bovine γ-globulin injected into laying hens, indicate that this protein can pass from the maternal circulation into the yolk of maturing ova. Electrophoretic and ultracentrifugal properties of the molecules are unchanged following the transit.

A second series of experiments, wherein bovine γ-globulin injections were made into the yolk of developing chick embryos, showed great variation in the percentage of embryos having foreign protein in their circulatory system. Recent experiments demonstrate that during, or following, the injection procedure, leakage of the bovine γ-globulin into the allantois and extra-embryonic coelom occurs. It then enters the circulatory system by passing through the cell layers lining those cavities. The bovine protein does not pass through the endodermal lining of the yolk sac, since it is never found in the circulatory system following either ventral injection into yolk or by maternal injection as in the early experiments. What the difference in permeability between the yolk sac and the extra-embryonic coelom wall is due to is still unknown.

The evolutionary implications of these findings are rather interesting. Apparently there is little specificity with regard to what proteins pass into

the yolk; instead, at a later stage, the embryo possesses its own defense mechanism by having a selectively permeable lining surrounding its nutrient supply.

N. K. W.

April 8, 1959

IMMUNOLOGICAL ENHANCEMENT. By Nathan B. Kaliss, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

Mice of an inbred strain will reject skin grafts from donor mice of a different strain. A second graft from the same donor will be rejected more rapidly than the first, indicating that the recipient has been immunologically sensitized to antigens of the donor. If, however, cells of a tumor are transferred from donor to recipient, growth of a subsequent inoculation from the same tumor is often enhanced. Enhancement may also occur if, instead of initially being injected with the tumor, the host is injected with antiserum elicited against another animal which has been exposed to the tumor. In the former case the ability to enhance tumor growth is permanent, whereas in the latter it is only temporary. In both cases, circulating antibody must be present if enhancement is to occur Injection of cortisone, which inhibits the production of circulating antibodies, also inhibits enhancement of tumors by mice which could otherwise do so.

The phenomenon of enhancement appears to be related to that of immunological sensitization. If the second injection of tumor is made at a certain time interval (5-7 days) after the initial inoculation, the tumor is rejected rather than enhanced. On the other hand, enhancement has recently been observed with grafts of noncancer cells.

Several hypotheses for enhancement are considered. One is that the immunological reaction of the host against the implanted cells is somehow blocked by the implant. A second is that cells resistant to host antibody are selected, and multiply in the host. Both of the above are considered unlikely, and a third view is put forward, that under the influence of antibody a physiological change is elicited in the tumor cells, which may lead to enhanced tumor growth.

J. FRANKEL

April 15, 1959

IN VITRO CULTURE OF MARINE SYMBIOTIC ALGAE. By John J. A. McLaughlin, Haskins Laboratories, New York, New York.

The long-term question, "What are zooxanthellae?" has recently been solved. These organisms which inhabit the gastrodermis and mesoglea of coelenterates, as well as tissues of many other organisms, have been described in the past as gametes, parasitic algae, unicellular glands, and zoochlorellae.

Upon extraction from anenomes and *Cassiopeia* and transfer into unialgal, bacteria-free cultures, however, it has been shown that the zooxan-

thellae are dinoflagellates which undergo a life cycle containing both free-swimming and vegetative phases. Freudenthal has studied the life cycle in which the free-swimming form of *Gymnodinium adriaticans* loses its flagella, sulcus, girdle, and nucleus, and develops chlorophyll bodies and a large orange assimilation product. By this stage, the cell is vegetative, larger in size, and round in shape. In the next stage, called *Bleeching Out*, the cell wall thickens, the chlorophyll disappears, and within the cell arises a *cytoplasmic basket* containing moving bodies. These bodies leave through a pore in the cell wall and develop either into the free-swimming form, or into an early form of the vegetative cell. The zooxanthellae observed in animal tissue are predominately in the vegetative form.

The exact ecological relationship of host and "symbiont" has not been determined. However, nutritional studies in unialgal, bacteria-free cultures indicate that zooxanthellae can utilize such breakdown products of protein metabolism as urea, taurine, and ammonia. Since tropic waters are low in phosphate and nitrate, a host containing these compounds offers a richer environment for the zooxanthellae. In return, the zooxanthellae in the animal tissue produce oxygen and carbohydrate which may be of use to its host. Recent experiments by Cadet Hand indicate that C_{14} from CO_2 in sea water eventually is taken up by the anenomes in the region of the zooxanthellae.

As a result of these studies, the zooxanthellae have been accurately identified. *In vitro* biochemical experiments are now elucidating the metabolic processes which may be instrumental in symbiosis.

SUSAN D. TENNEY

NEUROLOGICAL STUDY UNIT

April 12, 1959

EPIDEMIOLOGIC INVESTIGATION OF CHRONIC NEUROLOGIC DISEASES. AMYOTROPHIC LATERAL SCLEROSIS IN GUAM, KURU, AND MINAMATA DISEASES. By Leonard T. Kurland, National Institute of Neurological Diseases, Bethesda, Maryland.

Epidemiological methods have been applied to the study of four chronic neurological diseases, multiple sclerosis (MS), amytrophic lateral sclerosis (ALS), Kuru, and Minamata disease. The first two were compared as to geographical incidence in the U. S. and Canada with the principle finding that MS is increased in the North, whereas ALS is evenly distributed. They therefore would seem to be discrete diseases. ALS is in fact evenly distributed throughout the world's populations with the sole exception occurring on the Pacific island of Guam. It is 100 times as prevalent among the island's Chimorro tribesmen as throughout the rest of the world with 8-10 per cent of the native population dying of the disease. The Navy personnel on the island are unafflicted. One may speculate that the disease among the Chimorros is similar to or identical with one of the hereditary forms of ALS.

Kuru is a recently described disease which is unknown beyond the native populations on New Guinea. It accounts for 50 per cent of their

deaths and occurs with a distribution reminiscent of that of pellagra as it used to occur in the South, i.e. primarily limited to women and children. Occasional wives captured from other tribal groups have developed the disease, suggesting a nongenetic origin. Despite exhaustive investigations no etiology has been found.

Minamata disease is found among fish-eaters living in and near the city of Minamata on the southern island of Japan. It is characterized by ataxia, dysarthria, paresthesias, spasticity, intellectual and emotional deterioration, and may end with inanition and death. Its cause has been traced to the eating of fish, especially shellfish, which harbor an unidentified toxin. The disease was first recognized in 1943, approximately coincident with the shifting of effluent chemical wastes from the open sea into the fishing waters of the bay.

N. R. M.

BOOK REVIEWS

CHOLESTEROL. By David Kritchevsky. New York, John Wiley and Sons, Inc., 1957. 291 pp. $9.75.

The extent of scientific literature on the subject of cholesterol prompted the author of this book to bring together in one volume a review of all the various aspects of this remarkable compound. The result is an imposing summary of the chemistry, biochemistry, and physiology of cholesterol, from the cholesterol content of the brains of Egyptian mummies to the ability of cholesterol to promote the growth of the cockroach. Little available knowledge has been omitted, yet the length is reasonable and the text readable.

There are seven chapters, one each on the chemistry, biosynthesis, absorption and transport, metabolism, cholesterol in disease states, blood cholesterol, and analysis of cholesterol. Each chapter begins with an excellent historical review, progressing to present status of knowledge, the whole given unity by the author's own summary of unsolved problems and at times rather discerning impressions about possible directions for future research. The chemistry is clarified by the inclusion of many structural formulae. Liberal use of tables has made possible the presentation of much quantitative material. An exhaustive list of references concludes each chapter. Unfortunately the references are given in the order in which they occur in the text, with no grouping by subject matter, thus limiting the potential usefulness of these listings.

The chapters on chemistry and biosynthesis are thorough and complete. The treatment of the clinical problems and applications is somewhat superficial, but the inclusion of extensive references to the literature compensates for this deficit. A summary of the current status of cholesterol lowering agents is included. Additional information in the appendices includes the physical constants of cholesterol, cholesterol content of various foods and tissues, and serum cholesterol concentrations of many species.

To everyone interested in cholesterol this book will be of great value as a reference to all aspects of the subject. The author has done a mammoth task of condensing knowledge without omission of important information.

MARGARET J. ALBRINK

EMBRYONIC NUTRITION. Dorothea Rudnick, Ed. Chicago, University of Chicago Press, 1958. xi, 113 pp. $3.25.

Six papers presented at one of the 1956 Developmental Biology Conferences compose this volume. Each is a review and critique of the present status of the respective investigator's research field (the authors being E. J. Boell, J. L. Kavanau, S. Smith, J. D. Ebert, N. T. Spratt, and F. Moog). Unfortunately, several of the papers are reiterations of experimental work and ideas that have been presented before in review papers. There hardly seems to be justification for republishing some of this material, especially in view of the fact that there is no closely knit organizational relationship between the subjects discussed at the conference that might preclude omission of one or two papers. Despite these drawbacks the book must be rated as a worthwhile contribution to the developmental biological literature because it does con-

tain some outstanding discussions and ideas that may become central to our theory in future years.

Of interest is a concept that Kavanau and Smith touch on in completely different ways. Too often biologists interested in "the inducer" or other control substances, forget the over-all integrated pattern of the epigenetic system in space and in time. What happens at any one instant in ontogeny is wholly an automatic result of the sequence of events that led up to that moment, events such as the attainment of a variety of structures, of a metabolic apparatus, or of metabolic concentrations. When any one stratum or spectrum is reached, the next must automatically follow unless some abnormal external factor alters the system. Thus the mature ovum with its multitude of specific inclusions arranged in certain ways is given the initial stimulus at fertilization—reactions occur because of the distribution of the materials and because of their properties—stratum follows stratum, and the adult is attained. Because of the necessity for categorization in thinking about, and in attacking experimentally, this dynamic system, biologists have somewhat unconsciously accepted the idea that many independent reactions may be strung together to produce the over-all complexity. As a result much work is directed toward discovering the control mechanisms of various steps in ontogeny (as "inducer"). The danger in such work is obvious; it is absolutely essential that experimental results be interpreted in relation to the whole embryo as it develops in time—the control mechanism of any one aspect is an abstraction that must never be given anthropomorphic connotations as is often the case.

The bibliographies of the papers are extensive and cover in excellent fashion the literature of each subject up to late 1956. The binding and paper are of high quality and serve to enhance the value of the book. These attributes, however, do not outweigh the fact that much of the material presented is available elsewhere. The main value, then, is in the gathering together of diverse information all related to embryonic nutrition. The book is perhaps best fitted for inclusion in biological libraries.

N. K. W.

IMMUNOLOGY AND DEVELOPMENT. Mac V. Edds, Jr., Ed. Chicago, The University of Chicago Press, 1958. 59 pp. $2.50.

Occasionally when perusing the literature of science, one encounters a volume that might serve as a model for all authors—such a book is this report of one of the Developmental Biology Conferences held in 1956. Dr. Edds, writing in his characteristically lucid and erudite fashion, has prepared an essay treatment of the title subject. Individual reports by the conference participants are integrated into cohesive treatments of the aspects of immunology that have been applied to developmental problems.

To date, the information gained by immunological techniques can best be classed as descriptive embryology. Identifying and tracing various organ or tissue specific antigens through ontogeny has added another dimension to our knowledge of the epigenetic system. However, it is too often forgotten that such procedures do not provide any definite evidence on casual relationships between parts of the developing system, nor do they elucidate the

physiological prerequisites for the formation of the very antigens studied. Instead, the immunochemist deals with differentiated end products of what is most probably a complex series of events in time and space.

Much evidence does support the view that at least part of the specificity characteristic of organ differentiation is associated with immunological properties. If this is so, then specific organs might interact with homologous ones and thereby effect their differentiation or growth; the interaction occurring perhaps via immunochemically identifiable substances. Although absolutely no definitive evidence is available on this problem, several hypotheses have been advanced to deal with it, the best known being the Template-Antitemplate of Weiss. No satisfactory experimental test of any of these hypotheses has been designed; most probably one will not be until new techniques are available.

Perhaps the most valuable aspects of Edd's treatise are the discussions of currently unproved hypotheses, ideas, and disputes. By phrasing the problems now confronting developmental immunologists so candidly, he has not only done them a service in clearing the air for future progress, but also has provided a panoramic view of the whole field for the nonspecialist, a view that will enable such a person to read forthcoming work critically and put it in correct perspective. Every developmental biologist will profit from reading this volume; it is highly recommended.

N. K. W.

THE PHYSICAL FOUNDATION OF BIOLOGY. By Walter M. Elsasser. New York, Pergamon Press, 1958. x, 219 pp. $4.75.

Recent biological thought has been considerably influenced by several theoretical contributions of nonbiologists, such as Schrödinger's *What is Life?* and Wiener's *Cybernetics*. Whether the present book will take its place among these classics is problematic, for there are important differences between it and past efforts. The purpose of the book is to bring to bear on general biological theory the disciplines of quantum mechanics, information theory, and automation. The outcome is perhaps less important than the approach.

The first half of the book is devoted to excellent nonmathematical explications of feedback, information, and information storage. It is interspersed throughout with remarks emphasizing the great extent to which mechanistic devices can mimic biological systems. But these similarities in no way imply that organisms do actually function as machines. The second half is devoted to the thesis that, in part, organisms do not behave mechanistically.

Specifically, the author sets forth the working hypothesis that "organisms seem to behave as automata in all respects, except for information storage." There exist conditions of long-term stability in living systems which are unaccountable in quantum-mechanical principles; this stability is said to be describable in terms of *biotonic laws* which "express the conditions for the stability or reproduction of information in the organism in the absence of mechanical storage." The author's stand is in no sense of the word vitalistic; the biotonic laws are to be causal (in a wide sense), and operationally accessible.

Elsasser is careful to state that the deduction of such emergent properties "should be based on observed *explicit discrepancies* between the way in which a mechanism is expected to operate and the way in which the organism is actually found to behave." Unfortunately, it is just in the formulation of explicit discrepancies that the argument is least convincing. The main point to be got from the analysis is that the author finds it difficult to believe that sufficient information can be stored over long periods of time in molecular sructures in the face of the relatively enormous noise from random processes at the microscopic level. Now, this is a quantitative argument: the biologically trained reader will doubtless pick out many misconceptions in the author's' treatment of biological phenomena, a great many of which, when corrected, provide quantitative counterarguments. None of these can, of course, be any more decisive than the author's original conjectures without vastly more quantitative data than can be had at the present time. Nevertheless, the over-all effect with respect to explicit discrepancies is to fail to convince.

To over-emphasize the last point would, however, be grossly unjust. For the unique and vastly important property of Elsasser's conjectures is that, by their quantitative and operational nature, *they are in principle testable.* Thus they cannot be overlooked by anyone seriously interested in the foundations of biology. The outcome is less important than the approach.

Elsasser also presents the sketch of a remarkable theorem indicating that biotonic laws cannot be proved incompatible with quantum mechanics. This result is based on Bohr's principle of generalized complementarity, and a "principle of finite classes" such that the number of organisms is so small relative to the number of possible microscopic configurations of their components, that the "membership of the class of organisms will be exhausted before a statistical proof of contradiction can be achieved."

The difference between this book and the previous classics will now be apparent. The latter gave the biologist the feeling (real or not) of a sounder foundation for the mechanistic hypothesis. Elsasser attempts to unsettle that foundation somewhat, and for that reason may be somewhat less "popular." But, regardless of the fate of biotonic laws, this book is a lucid, rigorous, and stimulating prototype of the manner in which such controversial and inaccessible subjects should be handled.

F. M. W.

THE CHEMICAL PREVENTION OF CARDIAC NECROSES. By Hans Selye. New York, The Ronald Press Company, 1958. 235 pp. $7.50.

In keeping with his well-known ability to classify and organize a vast amount of information, Dr. Selye has summarized the results of his own studies and those of other investigators that pertain to experimental myocardial necrosis. Although the title of his monograph suggests prophylaxis, a large part of the book deals with mechanisms of production of experimental "cardiopathies."

Rats were used for most of the author's experiments. The animals were "conditioned" by the daily administration of a synthetic adrenal steroid that possessed both glucocorticoid and mineralocorticoid properties. Cardiac

necroses sometimes resulted with the steroid "conditioning" itself. An increased incidence of the necrotic changes in the myocardium was produced by dietary supplements of various sodium salts, especially sulfate, phosphate, and perchlorate. Whereas sodium salts predisposed to or enhanced the production of myocardial necrosis, potassium and magnesium salts protected the myocardium from damage. The anionic constituent of the sodium, potassium, or magnesium salt used was found to be an important factor in determining the activity of a given compound. "Conditioning" was also attempted by the use of other steroids. Simple stresses were found to increase the incidence of myocardial necroses in steroid-treated rats. A portion of the monograph deals with re-interpretation of the previously reported experimental and clinical findings of others in the light of Dr. Selye's information.

The author concludes that steroids may "condition" the animal heart for the development of necroses following exposure to various sodium salts or nonspecific stresses. He describes a resemblance of the experimentally produced lesions to such disease entities as Fiedler's myocarditis, and myocardial infarction without coronary occlusion. The fact that certain salts of potassium and magnesium were found to protect the rats from the experimental lesions raised the possibility to Dr. Selye that future therapy of clinically observed myocardial necroses might include the use of these electrolytes.

Readers who dislike multiple titles and subtitles that break up the continuity of their reading may find Dr. Selye's monograph trying in its carefully outlined style. The lesions produced by the author in "conditioned" rats bear a marked resemblance to the lesions of hypokalemia. The studies are not complete and the exact pathogenesis of the necroses has not been clarified. Although the author states that "there is no evidence to prove that the same labile biochemical system that is so readily deranged by corticoids and electrolytes in animals also exists in man . . . ," throughout the book he repeatedly refers to clinical situations, and by such associations appears to be a salesman of the idea that the experimental and the clinical are one and the same. This concept, however unacceptable, is a stimulating feature of the monograph to the clinically oriented reader.

M. JAY GOODKIND

THE CHEMISTRY OF THE STEROIDS. By W. Klyne. London, Methuen & Co., Ltd.; New York, John Wiley & Sons, Inc. 215 pp. $3.50.

This book, although small in size, will prove of great value to anyone desirous of obtaining a good background in steroid chemistry. Klyne's purpose in writing this book was to provide (1) an outline of the chemistry of the steroids, (2) a meeting ground for the nonchemist, medical investigator, and the steroid chemist, and (3) a number of helpful references from the gigantic and ever-increasing list of publications in this area. This work is thoroughly integrated; numerous *and* necessary cross-references appear throughout the book, and one immediately gets the idea that Sir Charles Sherrington's physiological concept of "life in little" is beautifully extended in literary form.

Chapter one contains an exciting outline of the history of the steroids. That the author is very familiar with the subject and its literature is made very obvious by his knowledge of the so-called "classical period" (1900-1932). During this time Profs. Wieland and Windaus of Germany demonstrated, through immensely painstaking and step-wise degradations and determinations of ring size, the molecular "old" formulae of a few common compounds, for which they received the Nobel Prize in 1928. The first hint that Wieland and Windaus' laboriously deduced structures for cholic acid and cholesterol might be wrong came from the isolation of Diels' hydrocarbon by selenium dehydrogenation of cholesterol, and from the x-ray studies of Bernal which indicated that a molecule having the "old" structure could not fit into the crystal ("It would have been too fat at the point where there is [was] a quarternary carbon atom, C-9."). Rosenheim and King, working in London in 1932, suggested that the classical work, together with the formulae of Diel's hydrocarbon and Bernal's x-ray data, could be represented in terms of a formula as is now used, a five-membered ring D. This structure suggested almost simultaneously by Wieland, proved to be correct, and all of the classical results were easily translated into the new structure. (The story is told most graphically by King in an Obituary Notice of Rosenheim in *J. chem. Soc.*, 1956, 800.) The "early modern period" (1932-1947) followed with the elucidation of the sex hormones by Butenandt, Ruzicka, and Marrian, and their preparation from inexpensive cholesterol. Cortisone and many related, but inactive, compounds were extracted from the adrenal cortex during this same period by Reichstein, Kendall, and Wintersteiner. The "later modern period"—or the present time—is characterized by the unfolding story of the newer adrenal corticosteroids (the isolation of aldosterone and the chemical modification of cortisone and hydrocortisone), the total synthesis of the steroids by Sir Robert Robinson of Oxford and Dr. R. B. Woodward of Harvard, as well as the introduction of a whole new and exciting discipline in the clinical analysis and interpretation of blood and urinary steroids.

Chapter 2 presents a literary *tour de force* on the stereochemistry of the steroids, conformations, nomenclature, and total synthesis. Chapters 3, 4, and 5 are primarily concerned with the physical and chemical properties (simple and complex functional groups) of steroids. In addition to providing information, the author presents a rather interesting account of the methodology involved in obtaining same. The data on the infra-red spectra of steroids are quite informative and easy to comprehend. Likewise, the sections on optical rotations, melting points and solubilities are written with clarity and contain much useful information. With all of this much needed background, the author clears the way for the book's crowning achievement: chapters 6, 7, and 8 on the naturally-occurring steroids (sterols, vitamin D, trimethylsterols, the bile acids, the hormones and related steroids, the cardenolides, bufadienolides, sapogenins and the steroid alkaloids). The section on the oestrogens, androgens, progesterone and the adrenocortical steroids is the most accurate, remarkable, and enjoyable I have ever read. The only thing missing is the very recent and meaningful work of Dr. J. Zander on the isolation of Δ^4-3-keto-pregnene 20 alpha and 20 beta-ol and Dr. T. R. Forbes' biological characterization of these ovarian gestagens

(*J. clin. Endocr.*, 1958, *18*, 337; *Klin. Wschr.*, 1957, *35*, 143). Klyne's manuscript was undoubtedly in press when this work appeared. His thoroughness leads me to believe that it would be difficult for him to miss one reference in a thousand.

The last chapter (9) on the separation and identification of steroids provides a good analysis of the methods involved, their limitations, and a rather large number of helpful hints for both the beginner and the professional. This work merits serious consideration for use as a required textbook in elementary biochemistry, laboratory problems in steroid chemistry, and as an introduction to the study of steroids. This is one of those useful books that will help to make the coming era of what I should like to call *molecular medicine* an immediate reality.

JOHN THOMAS VELARDO

OBSTETRIC AND GYNECOLOGIC MILESTONES: ESSAYS IN EPONYMY. By Harold Speert. New York, Macmillan Co., 1958. ix, 700 pp. $15.00.

Scholarly enthusiasm is infectious, and Dr. Speert has been able to communicate to the reader much of his delight in pursuing the historical and biographical aspects of almost 80 eponyms in obstetrics and gynecology. The range of names, places, and periods is wide—from Gabriele Falloppio in sixteenth century Padua to Joe Vincent Meigs in twentieth century Boston. Not every schoolboy knows the history of the Trendelenburg position, Mauriceau's manoeuvre, or Nabothian cysts. In fact, it was with some surprise that this reviewer learned that the latter are not named in honor of the grapes of Naboth's vineyard (I Kings *21*) but for Martin Naboth, professor of chemistry in Leipzig in the early decades of the eighteenth century. Scholarship is combined with a felicitous style, and the choice of illustrations is in keeping with both. This is a book which every scientist interested in the female reproductive organs will enjoy savoring at his leisure.

WILLIAM B. OBER

BIOCHEMICAL PREPARATIONS. Vol. 6. Carl S. Vestling, Editor-in-Chief. New York, John Wiley & Sons, Inc., 1958. 105 pp. $5.25.

The aim of this series of annual publications is to provide preparative methods frequently needed in biochemical research. There is a discriminating selection of compounds whose inclusion would be most widely useful, and a good choice of methods for their preparation. The presentation is exceptionally lucid.

The compounds selected include enzymes, substrates, and cofactors, from all fields of biochemistry. Some compounds chosen are available commercially, but, for a variety of reasons, methods are still needed for their preparation. Thus new methods for isolation of RNA and DNA are included, which minimize degradation occurring during extraction. Isolation of very small amounts of insulin by fibril formation is described; this, however, apparently requires considerable skill, and no mention is made of paper chromatographic methods, which have proved successful in several laboratories. The book

jacket lists cytochrome c^1. Disappointingly, this must be interpreted, not as crystallization of cytochrome c_1, an especially enigmatic cytochrome not yet obtained in crystalline form, but as crystallization of cytochrome c, see footnote one! Cytochrome c containing 0.45 per cent iron is isolated by direct resin treatment of crude tissue extracts, omitting the preliminary isolation of 0.35 per cent iron cytochrome, as described in volume 5. Muscle phosphorylase "a" is now prepared from the "b" form, rather than by the direct isolation described in volume 3, and controlled addition of calcium ions replaces the previous method, which unknowingly depended on extraction of metal ions from filter paper.

The methods chosen for description are authoritative and reliable, and have been checked in laboratories other than those of the submitter; occasional notes by the checkers suggest slight modifications of technique, or record different yields. Many procedures are for isolation from biological material, but some compounds, including phosphoserine, are prepared by chemical synthesis, and others, for example ribulose diphosphate, by enzymatic synthesis. Preparation of the range of compounds covers a variety of techniques, including preparation of acetone powders, zone electrophoresis on starch, and low temperature ethanol fractionation; this is in keeping with the aim of providing experience for students.

Unity is given to a book written by many contributors, by adopting the same plan for each chapter. The first section of each is a concise outline of principles involved in the preparation. There is then a brief discussion of the starting materials. The procedure is described in sufficient detail for its accomplishment without reference to original publications; the last sections briefly describe the properties and purity of the products, and review alternate methods of preparation.

A series of volumes presenting well-tested methods for the preparation of a wide range of compounds is obviously highly desirable. This need is excellently met by *Biochemical Preparations.*

VALDA M. CRADDOCK

Ciba Foundation Symposium on the Cerebrospinal Fluid. PRODUCTION, CIRCULATION, AND ABSORPTION. Edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. Boston, Little, Brown, and Company, 1958. 335 pp. $9.00.

The production, circulation, and absorption of the cerebrospinal fluid remains one of the great riddles confronting students of the nervous system. This symposium consists of fifteen presentations of various aspects of the problem by a group of authors representing many disciplines. Their approaches to the subject include many experimental techniques as well as clinicopathological observations of patients with hydrocephalus. The discussion following each paper adds considerably to the reader's perspective of each topic.

The choroid plexus is the subject of a good deal of attention. Kappers' observations on human embryos disclose that the plexus goes through three phases of development, the second characterized by the storage of glycogen in the choroid epithelium, the significance of which is as yet unexplained. The

well-illustrated presentation of electron microscopy of the mammalian choroid plexus by Wislocki and Ladman discloses many fine details of intracellular structure, some of which suggest strongly the presence of an active secretory process. The tissue culture experiments of Lumsden show that under the experimental conditions the choroid villus is capable of absorbing and concentrating dye solutions and maintaining a pH gradient. Other anatomical studies deal with structural details of choroid plexus innervation, arachnoid villi, nerve root sheaths, and the perivascular and supposed tissue spaces of the brain.

Of greatest interest to this reader were the various papers and discussions relating to radioactive isotope studies of the cerebrospinal fluid. The statements of Selverstone are of great value in dispelling the confusion which has arisen from false interpretation of observations made in earlier isotope work —unwarranted conclusions which seemed to invalidate the classical concepts of Weed. Bowsher has demonstrated a higher sodium concentration in CSF collected directly from the choroid plexus than in free ventricular or cisternal fluid, and suggests that osmotic dilution of this initially hypertonic fluid is the mechanism of CSF formation. Unfortunately the number of observations is small, but further work may put this theory on a firmer basis.

Dott and Gillingham suggest that localized expanding arachnoid pouches in communication with the general subarachnoid space may be formed by the pumping action of pulsating cerebral arteries in the region, a concept which is not quite convincing. Other papers of a clinical or pathological nature do little more than re-explore familiar ground.

This symposium succeeds in presenting diverse current ideas about the riddle of the cerebrospinal fluid, raises many interesting questions, answers few.

SAUL A. FRANKEL

ADMINISTRATIVE MEDICINE. Transactions of the Fifth Macy Conference, October 29, 30, and 31, 1956. George S. Stevenson, Ed. New York, Corlies, Macy and Company, 1958. 197 pp. $3.75.

This book of conversations (Transactions) is focused on presentations of three experts in the field of mental health: Lemkau, Bay, and Rees. Eighteen additional experts from mental health and other fields of human relations (health, political science, sociology, and labor) were participants in the three-day conference in 1956 at Princeton, New Jersey. The editing is superb and must have been most difficult.

The belief of the Macy Foundation is that such conferences can stimulate a free exchange of ideas, reduce prejudice, and broaden perspectives of persons in medicine and allied disciplines. The Foundation also believes that such conversations are often disconnected and confusing. The conversations in this book are consistent with these beliefs.

Around the focus of three brief informal presentations of Lemkau, Bay, and Rees, there was a lively discussion which could be put together in several ways. The following is one such synthesis. The participants seem to be saying that society has locked large numbers of its mentally ill in "mausoleums" and has done little to treat, or rehabilitate, its mentally ill or to

prevent mental illness in the first place. The assumption is made that this way of dealing with the large, complex problem is unsatisfactory, and that sweeping changes in mental health programs and the care of mentally ill are indicated for the benefit of all. The "open door" policy of all mental hospitals is advocated and discussed. Community mental health programs are presented which include provisions for in-patient and out-patient services for all community people when indicated. The program so outlined is thought about in terms of use of the local general hospital, family physician, and public health nurse as the providers of community health services which would be concentrated in the family rather than about a single individual patient. The problems of mental health workers (family physician, public health nurse, consulting psychiatrist, and local hospital) would become those of families and would involve mental and other illness directly, and indirectly employment, housing, sanitation, education, etc. Finally, in such a program the remote "mausoleums" which now store so many of the mentally ill would close from lack of patients.

An extensive discussion occurred about desirable changes which are resisted by the status quo in mental hospitals as well as in the community. Different mental health administrators and policies would need to develop, and an informed community regarding mental health would need to become real in order for the mental hospital to become an "integrated" (community) hospital rather than a remote, locked storage place. Time, patience, extensive effort, and perhaps good luck would seem important for such a realization. Rees seems to have shown that much can be done, though his conversation seems to laud his own program too much to make his situation real.

The conversations are stimulating and the ideas are refreshing and germane to the whole field of administrative medicine, but there is some confusion and conversation domination by some participants. This reviewer had the feeling that much more could have been learned by hearing more from some participants such as Simons (sociology), and Ascher (political science). Brindle (United Auto Workers) was present but said little or nothing. What does he think of all this—one would like to know. Also, one would like to know how the families, public health nurses, and family physicians might respond to the thoughts of the leaders in this field. They, the former, are the persons finally implicated to face the problems outlined.

This book is well worth an evening of reading, and a longer period of thought, for those interested in health and community planning.

R. DUFF

PSORIASIS. By Peter Flesch, *et al.* Annals of the New York Academy of Sciences, vol. 73, art. 5, pp. 911-1037. New York, 1958.

On May 10, 1958 a conference on Psoriasis was held by the New York Academy of Sciences. The papers presented at that conference resulted in the monograph reviewed here. Dr. George Hambrick, Jr., from the University of Pennsylvania, discussed the clinical features of psoriasis, a chronic, hyperkeratotic skin disease of unknown etiology that has piqued the curiosity of physician and layman alike since it was first described by Celsus.

The erythematous psoriatic lesions, covered by mica-like scales, may be solitary or generalized. Although spontaneous remissions sometimes occur, for the most part the disease is recurrent and deforming. In 6 per cent of the patients psoriasis is complicated by rheumatoidlike arthritis involving primarily the terminal interphalangeal joints of the hands and feet which may be incapacitating.

Dr. Elson Helwig of the Armed Forces Institute of Pathology discussed the histopathological changes noted in more than 500 cases of psoriasis. Dr. O. Braun-Falso of Johannes Gutenberg University in Mainz, Germany considered the histochemical findings in psoriasis, particularly the localization of inorganic substances, carbohydrates, lipids, proteins, amino acids, sulfhydryl compounds, nucleic acids, and enzymes. The psoriatic horny layer with its parakeratotic cells, which represent incomplete cornification, contains considerably more sulfhydryl groups than the normal horny layer. This abnormality may point to a defect in protein metabolism with resultant abnormal keratinization. Dr. Daphne Anderson Roe of Vassar College reported an electrophoretic study of the proteins extracted from psoriatic scales. Chemical analysis of the water-soluble components of psoriatic scales was described by Dr. Peter Flesch and Dr. Elizabeth Esoda of the University of Pennsylvania. Dr. Allan Lorincz of the University of Chicago briefly discussed theories on pathogenesis of psoriasis. Dr. Simon Rothberg of the National Cancer Institute considered the significance of elevated arginase activity in psoriatic scales in relationship to the Krebs-Henseleit ornithine cycle. Dr. Alfred Bollets and Dr. Rachel Turner of Wayne University discussed psoriatic arthritis and gave a statistical comparison of rheumatoid arthritis and arthritis associated with psoriasis which emphasized their differences. Dr. M. H. Samietz of the University of Pennsylvania presented a thoughtful report on past and present therapy of psoriasis, pointing out the rationale—or lack of it—for various systemic and topical medicaments. Dr. J. Bleiberg of Beth Israel Hospital in Newark described results of treating patients with a new topical preparation. However, no adequate control system—in the sense of paired comparison or double blind study—was used.

In conclusion, this monograph represents a faithful recording of papers presented at a recent conference on Psoriasis. It is an up-to-date review of the known clinical, physiological, and pathological abnormalities of this disorder. In spite of the wealth of data already accumulated on psoriasis, much work remains to be done to discover its etiology.

MARGUERITE R. LERNER

ANIMAL DISEASE AND HUMAN HEALTH. Annals of the New York Academy of Sciences, vol. 10, art. 3, pp. 277-762. June 1958.

This publication is the result of a conference on *Animal Disease and Human Health* held by the New York Academy of Sciences in collaboration with the Communicable Disease Center, Public Health Service, Atlanta, Georgia, on September 11, 12, and 13, 1957. The subject matter considers the zoonoses which are "diseases communicable from animal to each other or to man, or as diseases due to animal parasites." The broad scope of the material presents

the problem as it relates to man in his health, history, culture, and economy. Many microorganisms including viruses, bacteria, fungi, metazoan parasites, and ectoparasites are dealt with in greater or less detail.

The monograph, containing 37 major contributions, treats several problems in eloquent fashion and might well serve as a major source of reference. These include: "Orphan Viruses of Man and Animals" by Hsuing and Melnick; "Human Antiviral Neutralizing Substances in Animal Sera" by Klein; "Leptospirosis"—an excellent treatise discussed in five papers; "Recent Developments in the Epidemiology of Rabies" reviewed in four papers; "Newcastle Disease" by Hanson and Brandley; "Cat-scratch Fever" by Prier. Most considerations include a description of the disease, pathogenic agent, epidemiology, vectors, pathology, and an approach to a solution of the problem both in the human and in the animal. It is comforting at times to find lengthy discussions of rather restrictive areas of other problems such as "New Knowledge on the Ecology of Sylvatic Plague" by Kartman, Prince, Quan, and Stark. The reviewer was also distressed by recurrent facts made by different authors in consecutive papers confined to a single agent. This treatment is unavoidable, however, and actually serves to enhance particular pertinent information. Many articles, although confined, supplement our current knowledge. Among these we might cite: "Listeriosis —a Potential Public Health Problem" by Winn, Cherry, and King; "Salmonellosis—Observations on Incidence and Control" by Edwards; "Animal Disease and Biological Warfare" by Hempy, and "Air-borne Transmission of Q fever: The Role of Parturition in the Generation of Infective Aerosols" by Welsh, Lennette, Abinanti and Winn. Each major section is prefaced with a concise introduction, and the entire volume is summarized in a formidable manner by Schuman.

The examples which have been used here do not necessarily imply that other worthy reviews or primary research reports with other agents are not available in the volume. Actually it should be emphasized that every article is relevant, and only the limitation of space prevents the mention of each.

The international agencies concerned with these public health problems are discussed in two articles both with regard to their organization and current programs. The concerted effort stimulated by these groups indicates that much progress is being made in the control and eradication of these diseases.

The health of man is related to his contact with the wild and domesticated species of animals. The latter groups he used for his comfort and gain but from which "he assumed the obvious hazard of sharing their diseases." On the other hand, Shahan and Traum point out that man may be the primary source of some diseases transmitted to lower animals. The interrelationships reside, therefore, in the "same continuum" from which the agent, the human, and the animal cannot and should not be "removed and studied as isolates" of "distinct categories of animal as opposed to human disease."

CARL J. WUST

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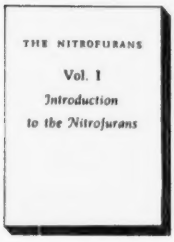

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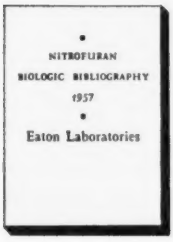

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